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PHENOTYPIC PLASTICITY, PARENTAL EFFECTS, AND PARENTAL CARE IN PLANTS? I. AN EXAMINATION OF SPIKE REFLECTANCE IN *PLANTAGO LANCEOLATA* (PLANTAGINACEAE)[1]

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We explore the relationships among phenotypic plasticity, parental effects, and parental care in plants by presenting data from four experiments examining reflectance/color patterns in *Plantago lanceolata*. In three experiments, we measured spike (inflorescence) reflectance between 362 and 850 nm using a spectrophotometer with an integrating sphere. Experiments show that (1) spike reflectance changes seasonally within and outside the visible portion of the spectrum of radiant energy, (2) increasing ambient temperature causes an individual plant to produce flowering and fruiting spikes that reflect more/lighten in color (the greatest changes occur in the regions around 550 nm and between 750 and 850 nm, the visible and near-infrared regions, respectively), (3) responses are reversible, (4) genotypes within populations and populations from different latitudes differ in mean reflectance and degree of phenotypic plasticity. In a fourth experiment, we measured internal spike temperature. Darker spikes, those produced at lower temperature, got hotter than did lighter spikes in full sun. Thus, plants can partially thermoregulate reproduction and the embryonic development of their offspring. In light of a previous experiment, data suggest that thermoregulation produces adaptive parental effects and is a mechanism by which *P. lanceolata* provides parental care.

Key words: North Carolina; parental care; parental effects; phenotypic plasticity; Plantaginaceae; *Plantago lanceolata*; reflectance; thermoregulation.

Phenotypic plasticity, parental effects, and parental care are each evolutionarily important phenomena. Phenotypic plasticity is a mechanism by which organisms are thought to acclimate to spatial and temporal environmental change (e.g., Schmalhausen, 1949; Bradshaw, 1965; Levins, 1968; Schlichting, 1986; Sultan, 1987; Futuyma and Moreno, 1988; Via et al., 1995; van Tienderen, 1997; Schlichting and Pigliucci, 1998). For this reason, it has become a major focus of empirical and theoretical studies of organisms living in spatially and temporally varying habitats (e.g., for plants: van Tienderen, 1991, 1997; Dudley and Schmitt, 1996; Winn, 1997; Donohue et al., 2000; van Kleunen et al., 2000; Sultan, 2001; Callahan and Pigliucci, 2002). Parental effects, the effects of parental phenotype on offspring phenotype that are transmitted independently of the genes that parents pass directly to offspring, are believed to influence the course of evolution for offspring traits whose phenotypes are influenced by such effects and for the parental traits producing the effects (e.g., Cheverud, 1984; Roach and Wulff, 1987; Kirkpatrick and Lande, 1989; Dudley, 1991; Jablonka and Lamb, 1995; Bernardo, 1996; Rossiter, 1996; Fox and Mousseau, 1998; Mousseau and Fox, 1998; Wolf et al., 1998). Such effects were once thought to be annoying sources of noise in quantitative genetic studies. Now a few empirical studies of animals show that parental effects can be adaptive (Mousseau and Dingle, 1991a, b; Sinervo, 1991, 1998; Fox and Mousseau, 1998). Comparable evidence for plants is still lacking (Donohue and Schmitt, 1998; Lacey, 1998; Mazer and Wolfe, 1998). Parental care in animals influences offspring fitness and is believed to be involved in the evolution of mate competition, altruism, and sexual selection (e.g., Clutton-Brock, 1991; Alcock, 2001).

Biologists studying animal behavior have long appreciated the intimate relationship between phenotypic plasticity, parental effects, and parental care. Parental care represents a special case of parental effects when parents modify their behavior in response to their environment and when this modification changes the environment of their offspring in a way that increases offspring fitness (Cheverud, 1984; Kirkpatrick and Lande, 1989; Lande and Kirkpatrick, 1990; Cheverud and Moore, 1994; Wolf et al., 1998). This phenomenon is illustrated in Fig. 1, which shows that maternal phenotype (Z_m) influences offspring phenotype (Z_o) and offspring fitness (w_o) indirectly by modifying offspring environment (E_o). All this occurs in addition to the direct contribution of maternal genes (G_M) to offspring genotype (G_o). If a trait associated with parental care has a genetic basis, then that trait's evolution can be influenced by its cross-generational effects on offspring fitness (e.g., Dickerson, 1947; Willham, 1963; Cheverud, 1984; Kirkpatrick and Lande, 1989; Lande and Kirkpatrick, 1990; Cheverud and Moore, 1994; Wolf et al., 1998). The multigenerational fitness of the parent (W_M) is determined by these cross-generational fitness effects and by the fitness effects expressed in the parental generation (w_M).

In contrast to biologists studying animal behavior, botanists have seldom examined the relationship between phenotypic plasticity, parental effects, and parental care. The primary reason is that biologists generally assume that plants do not provide parental care. Aside from discussions of the trade-off between seed (offspring) number and quality, we have found a negligible number of papers that mention parental care in

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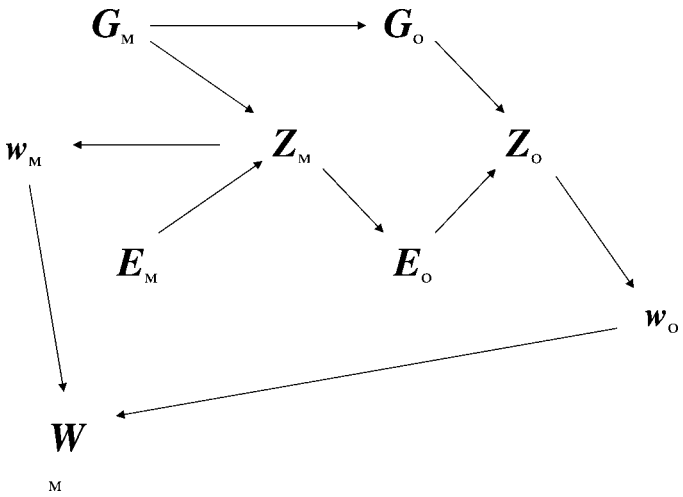

Fig. 1. The indirect effect of maternal phenotype (Z_m) on offspring phenotype (Z_o) and offspring fitness (w_o) through the parental modification of offspring environment (E_o). This pathway differs from that showing the direct effect of maternal genes (G_M) on offspring phenotype via offspring genotype (G_o). The multigenerational fitness of a parent (W_M) is determined by the fitness effects expressed in its own generation (w_M), e.g., progeny produced, and its cross-generational fitness effects (w_o).

plants (Wied and Galen, 1998; Galen and Stanton, 2003). Parental care may be more widespread in plants than has been recognized to date. The reason is that reproductive traits, which are generally viewed as influencing fitness in the parental generation through seed set, may also influence offspring fitness through their effects on offspring environment. One such phenotypically plastic reproductive trait may be the reflectance/color pattern in spikes (inflorescences) of *Plantago lanceolata*.

Here, in the first of several papers that address this hypothesis, we describe the phenotypic plasticity in spike reflectance of *P. lanceolata*. In four experiments, we looked at seasonal variation in spike reflectance (i.e., variation in serially produced spikes), tested the effect of temperature on reflectance, and explored the between- and within-population variation in reflectance and the effect of reflectance on floral temperature. Using the results of these experiments, we then discuss how phenotypic plasticity in reflectance could produce parental effects and how it could represent a parental care trait. The plasticity may allow parents to thermoregulate their own reproduction and the early embryonic development of their offspring in ways that improve offspring fitness and consequently also parental fitness.

MATERIALS AND METHODS

Biology of experimental organism—*Plantago lanceolata* L. (Plantaginaceae), ribwort plantain, is a short-lived perennial herb that grows in disturbed sites, abandoned crop fields, and lawns in temperate North America and in its native Eurasia. The species' biology is described in numerous publications (e.g., see Cavers et al., 1980; Antonovics and Primack, 1982; Wolff and van Delden, 1987; Kuiper and Bos, 1992; Lacey et al., 2003). Here we mention several features relevant to this study. Individuals grow vegetatively as rosettes. An individual rosette can produce side rosettes, but these remain very close to the original rosette. Most individuals used in our study grew at least 1 m apart; all grew at least 0.5 m apart.

With the onset of flowering, plants produce long-stalked spikes (inflorescences) from leaf axils. Protogynous flowers are subtended by bracts (modified leaves), which are the only parts of the flowers visible prior to flowering and which continue to be visible during flowering and most of fruit maturation. Flowering begins with stigma emergence from proximal flowers and progresses distally. After stigma emergence, petal lobes and anthers emerge. Petals persist during fruit maturation and form a broken translucent veil around the developing fruits on a spike. The duration of flowering and fruit maturation on a spike varies greatly from 1 wk to at least 5 wk, depending on the number of flowers per spike, ambient temperature, water availability, and probably also resource availability. In the North Carolina piedmont, flowering begins in late April and can continue into August, if water is available.

Reflectance measurements—Three of the four experiments involved quantitatively measuring reflectance of radiant energy both within and outside the visible (approximately 400–700 nm) range of the spectrum. Reflectance/color was measured using a Shimadzu spectrophotometer (Norcross, GA, USA) with an integrating sphere. The sphere is enclosed in a module that can be completely closed, which allowed us to measure percentage reflectance of an opaque three-dimensional object (a spike) when a narrow beam of light (width $= 5$ nm) is directed toward the center of the spike (width > 4 mm, length > 1.4 cm), which one places in the window of the sphere. The spectrophotometer measures reflectance of the area touched by the light beam. Initially, we measured percentage reflectance from 200 to 850 nm. However, because the change in reflectance from 200 to 370 nm was negligible across a sample of spikes that had developed at different temperatures, and because measuring reflectance below 360 was time-consuming, we limited our spectral scans to the range of 362–850 nm for our experiments. We measured reflectance at every 1 nm within that range.

We scanned spikes over this wide range to determine where in the spectrum reflectance might change seasonally, where temperature change might alter reflectance, and where populations and genotypes might differ in reflectance. Only by scanning over a wide range and by performing statistical analyses over the range could we achieve these goals. We replicated scans for each spike three times for experiments 1 and 2. Having observed that two replicates would suffice, we scanned each spike twice for experiment 3. Spikes were rotated slightly between replicate scans to take into account the heterogeneity in reflectance/color pattern over the spike surface. Mean reflectance value per nanometer was calculated for each spike per treatment and this value was used in statistical analyses.

In experiment 2, we scanned spikes at two developmental phases: preflowering and fruiting. Stigmas had not yet emerged from flowers in the preflowering phase, but would have emerged from proximal flowers in the next 2 d (E. Lacey, personal observation). Anther dehiscence had recently finished on fruiting spikes, and capsule swelling had begun. If anthers had not yet naturally dropped off, remnant anthers were gently removed before scanning fruiting spikes.

Experiment 1—*Methods*—Our first experiment measured natural seasonal change in spike reflectance/color pattern in a North Carolina population. On 13 April, we haphazardly collected and scanned 10 spikes, one per plant, from a small population in downtown Greensboro, North Carolina, USA. The population was located in a disturbed patch of land (approximately 5×3 m) bounded by a gravel parking lot, a driveway, and a four-lane street. One and 2 mo later, new spikes were collected and scanned ($N = 9$ for 12 May; $N = 10$ for 18 June). After that, spike production dropped, and new spikes fell below the minimum length (1.4 cm) needed to get a reliable scan. A 3-yr drought caused spike production to end early that year.

Results—Reflectance of a single spike changed greatly across the spectrum (Fig. 2). Generally, reflectance was low in the UV portion of the spectrum, it began rising at approximately 500 nm to a rounded peak at approximately 550 nm then declined gradually to approximately 680 nm, where it rose sharply to a high plateau, at about 750–850 nm. As the flowering season progressed, reflectance increased at all wavelengths. However, it increased most in the visible region of 500–680 nm and above 750 nm, the near-infrared region.

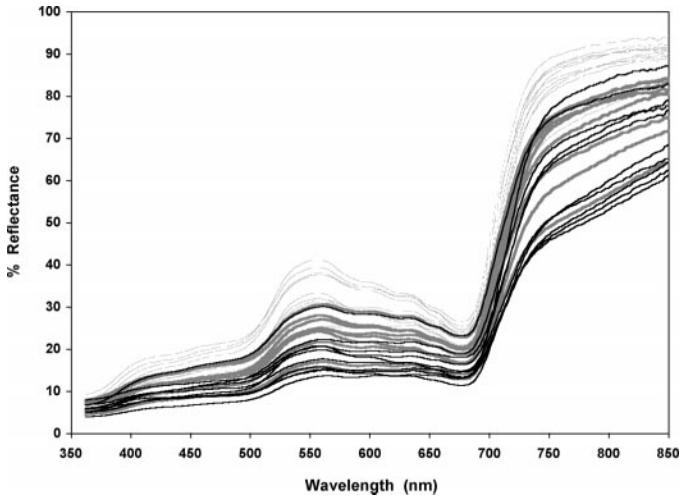

Fig. 2. Reflectance of preflowering spikes of *Plantago lanceolata* sampled from a natural population at three times during the reproductive season: 13 April = thin black lines, 12 May = thick grey lines, 18 June = thin grey lines.

Experiment 2—*Methods*—This experiment tested the effects of manipulating temperature and population source on reflectance pattern. It also tested whether or not change in reflectance is reversible. Plants from three sources were used: Bristol, Rhode Island (RI; 41.7° N), Charlottesville, Virginia (VA; 38.1° N), and Greensboro, North Carolina (NC; 36.1° N). We collected 20 plants from one population each in NC and in VA. The RI plants came from several populations in Bristol, but for the purpose of this experiment, all plants are treated as belonging to one population. Each plant was considered a unique genotype.

Genotypes were divided into two clones, potted, and grown for at least 1 mo under short days (26°C, 10 h day/20°C, 14 h night) to promote vegetative growth. They received half- strength Hoagland's solution once per week. The temperature was then reduced to 15°C day/10°C night. After an additional month, we transferred one clone of each genotype to a greenhouse, keeping the other in the growth chamber. Clones were spatially randomized in each location. At the time of transfer, the photoperiod was adjusted to 16 h day/8 h night in both locations. The growth chamber temperature remained at 15°C day/10°C night (our low-temperature treatment), whereas the greenhouse temperature was set at 22°C day/17°C (greenhouse-temperature treatment). As expected, the variance in temperature was greater in the greenhouse. Deviations occurred when temperature dropped below the settings on a few very cold days and rose above the setting on very hot days. All plants continued to receive half- strength Hoagland's once per week.

Flowering of the RI plants began in the growth chamber after 3 wk, followed by VA and then NC plants. The NC plants began flowering approximately 5 wk after onset of flower induction. In general, growth chamber plants flowered earlier than did greenhouse plants. When both clones of a genotype had preflowering spikes (or fruiting spikes), we scanned a single preflowering (or fruiting) spike from each clone.

When we finished the low- and greenhouse-temperature scans of fruiting spikes, we removed all visible spikes from the low-temperature (growth chamber) plants and raised the growth chamber temperature to 26°C day/20°C night (high-temperature treatment). Visible spikes were removed again 2 d later. After that plants were allowed to produce new spikes. Two weeks later, we began scanning preflowering and fruiting spikes that had developed at high temperature. After 1 mo, we again removed all existing spikes, lowered the growth chamber temperature to 20°C day/15°C night (medium-temperature treatment), and allowed new spike production at the new temperature. This treatment was included to see if individual plants would lower spike reflectance (i.e., darken spikes) if temperature dropped. Temperature setting resembled those of the greenhouse treatment. For the medium-temperature treatment, we scanned only preflowering spikes.

We grew clones in the greenhouse and growth chamber for several reasons. First, doing so allowed us to use spikes from clones that were at a similar developmental stage for the comparison of greenhouse- and low-temperature treatments. Also for this comparison, we eliminated any possible but unlikely error that might be introduced because of scanning day. Comparable spikes from both treatments were scanned on the same day. Third, we used spikes from the greenhouse- and low-temperature treatments for experiment 4.

Statistical analysis—Because we scanned the same clone per genotype for all growth chamber treatments but a different clone for the greenhouse treatment, we could not perform a single analysis to determine the effects of all four treatments and population on reflectance. Therefore, we analyzed three subsets of data: greenhouse + high-temperature treatments, greenhouse + low-temperature treatments, greenhouse + medium-temperature treatments. We performed two-way multivariate analyses of variance (MANOVA) and analyses of variance (ANOVA) (PROC GLM; SAS, 2000) on each data set, using treatment and population as fixed factors. Population was treated as fixed rather than random because the populations were not selected haphazardly or randomly. They were chosen to sample part of the latitudinal range over which the species grows in eastern North America. Type III sums of squares were used because samples sizes differed across treatments and source populations. Although we began the experiment with 20 genotypes from each population, a few clones did not flower and fruit in all treatments. Also, logistics associated with transporting plants to the spectrophotometer in a facility an hour away prevented us from scanning some clones in both developmental phases in all treatments. For each analysis, we used only genotypes for which we had data for both treatments considered in the analysis.

First, we conducted MANOVAs of reflectance pattern. We did this because reflectance values at different wavelengths might not have been independent of one another within a spike. Therefore, we tested whether or not populations and treatments differed in reflectance pattern over the whole spectrum, from 370 to 850 nm. The number of observations in each data set allowed us to use reflectance data for every 20 nm from 370 to 850 nm in each analysis, i.e., 25 values per observation. MANOVA produces multivariate F statistics for Wilks' lambda, Pillai's trace, Hotelling-Lawley trace, and Roy's greatest root. We report Wilks' lambda. For all of our analyses, the conclusions that one would draw from the values of the four statistics were identical. Second, we compared the populations (CONTRASTS statement) to see which populations differed from one another with respect to reflectance pattern. Third, we performed two-way ANOVAs at every 10 nm from 370 to 850 nm. The reason is that while MANOVA can test for population or treatment effects on reflectance pattern over the whole spectrum, it cannot identify the specific wavelengths where populations or treatments differ. We performed ANOVAs to identify these wavelengths, understanding that the results at neighboring wavelengths may be correlated with one another.

We also examined the effect of treatment and population on phenotypic plasticity in reflectance. Our measure of plasticity was the difference between reflectance at high and low temperatures at each wavelength for each growth-chamber clone. Large differences indicate high reflectance plasticity. Small differences indicate low plasticity. We used MANOVA to test the effect of population on plasticity pattern from 370 to 850 nm. If plasticity was found to differ between populations, we used two-way ANOVAs to identify the wavelengths where significant differences existed.

Finally, to assess how well reflectance in the preflowering phase predicts reflectance in the fruiting phase, we performed regression analyses on plants, regardless of origin, in the low- and high-temperature treatments (SAS, 2000). We regressed fruiting reflectance on preflowering reflectance at every 10 nm from 370 to 850 nm.

Results for preflowering spikes—Increasing ambient temperature significantly increased the overall reflectance of preflowering spikes (Fig. 3; Table 1 MANOVAs). The greatest temperature-sensitive changes occurred in a broad region around 550 nm and from approximately 750 to 850 nm, in the visible and near-infrared regions, respectively. Mean reflectance over all populations at 550 nm increased from 12% at low temperature to 26.7% at high temperature (122% relative increase). At 750 nm, it increased from a mean

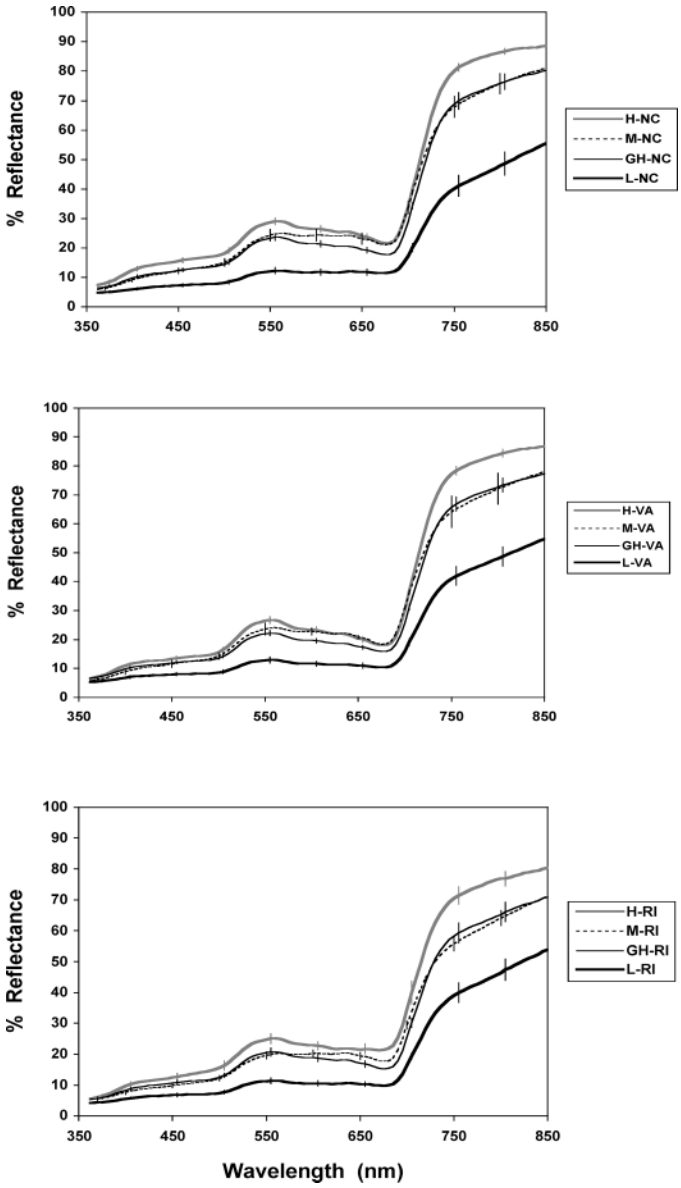

Fig. 3. Mean percent reflectance of preflowering spikes of *Plantago lanceolata* for four temperature treatments shown by population: North Carolina (NC), Virginia (VA), Rhode Island (RI). Temperature treatment: Low (L), Greenhouse (GH), Medium (M), High (H). The vertical bars show ±1 SE at 50-nm intervals.

of 40% at low temperature to 76% at high temperature (90% relative increase). However, even in the other regions, the treatment effects were often significant. The reflectance of greenhouse clones significantly surpassed that of low-temperature clones at all wavelengths (ANOVAs: all P values $< 1.0 \times 10^{-7}$). Reflectance of high-temperature clones significantly surpassed that of greenhouse clones at all wavelengths above 370 nm (ANOVAs: P value range: 0.00001–0.016). As expected, the reflectance patterns of the greenhouse and medium-temperature clones were most similar to each. These treatment groups differed only from 600 to 710 nm (ANOVAs: P value range: 0.0008–0.03).

Populations significantly differed from each other in reflectance pattern (Fig. 4; Table 1A), even when one lowers the critical P value to account for multiple comparisons (Table 1A contrasts; one exception = NC and VA populations in the GH + M analysis). The RI population reflected the least at all temperatures. At low temperature, the VA or NC population reflected the most, depending on wavelength. For higher temperatures, the NC population reflected the most. For the greenhouse + high-temperature data set, populations significantly differed at all wavelengths (ANOVAs: P value range: 0.00036–0.043) except from 660 to 710 nm. For the greenhouse + low-temperature data set, populations significantly differed in the region of 362–450 nm (ANOVAs: P value range: 0.0002–0.046) and at 670–680 nm (P values = 0.042 and 0.036, respectively). In the greenhouse + medium-temperature data set, differences were significant at all wavelengths above 362 nm (ANOVAs: P value range: 0.0047–0.043).

Although populations differed in mean reflectance, the MANOVAs detected no differences among populations with respect to reflectance plasticity (Table 1A: H + L data set). Significant treatment × population interactions were detected in two of the three MANOVAs (Table 1A). However, the ANOVAs did not detect any significant treatment × population interactions at any individual wavelength.

Results for fruiting spikes—The reflectance patterns of fruiting spikes differed from those of pre-flowering spikes (Fig. 5). The temperature effect was not as strong in the fruiting stage because of the dampening of the temperature effects around 550 nm and in the near-infrared region. In spite of these changes, the multivariate analyses showed that increasing temperature still significantly increased reflectance (Table 1B). At 550 nm, mean reflectance over all populations rose from 11.8% at low temperature to 21% at high temperature (78% relative change). At 750 nm, the mean rose from 34.7% at low temperature to 56.4% (63% relative change).

The NC, VA, and RI populations all significantly differed from each other in the fruiting phase (Table 1B, Fig. 6), even considering the multiple comparisons (Table 1B contrasts). Population differences were apparent in all temperature treatments (Fig. 6) and at almost wavelengths (for GH + L data set: $P < 0.0001$ for wavelengths <750 nm and $P < 0.05$ for ≥750 nm; for GH + H data set: all P values < 0.001). The RI population always reflected the least. At high temperature, the NC population reflected the most. In contrast, populations did not differ significantly in reflectance plasticity (Table 1B: H + L data set).

Overall, preflowering reflectance was not a great predictor of fruiting reflectance. The regression analyses of preflowering reflectance on fruiting reflectance at low temperature showed that the slopes of the regression lines significantly differed from 0 ($P < 0.05$) in the regions of 540–650 nm and 690–850 nm, that is, in the visible and near-infrared regions, respectively. However, the r^2 values in these regions were very low (range of r^2 values in the visible range = 0.10–0.11; range in the near-infrared region = 0.10–0.33). At high temperature, the slopes of the regression lines significantly differed from 0 ($P < 0.05$) at all wavelengths except in the region 640–720 nm. The r^2 values were higher (range = 0.27–0.61) but were still low.

Experiment 3*—Methods*—We examined genotypic differences in reflectance pattern and plasticity within two populations. We made three replicate clones of the nine NC genotypes and 14 RI genotypes used in experiment 2. New clones were grown in a greenhouse for an additional year under natural daylight and daylength to promote vegetative growth. We then randomly assigned the clones to positions in a growth chamber set at 15°C, 16 h day/10°C, 8 h night (low-temperature treatment). As plants flowered, we scanned one preflowering spike per clone. Then using the procedure described in experiment 2, we scanned spikes induced at 26°C day/20°C night (high-temperature treatment). Plants received half- strength Hoagland's solution twice per week in the growth chamber.

Statistical analysis—Each population was analyzed separately because we wanted to examine within-population genotypic variation and we had already determined in experiment 2 that populations differed. For each population, we conducted one-way MANOVAs to test the effect of genotype on reflectance (ref) pattern at high temperature (H), reflectance pattern at low temperature (L), and reflectance plasticity (ref_H–ref_L). The number of observations in each population allowed us to use reflectance values for every 40 nm from 370 to 850 nm in each analysis (i.e., 13 values per observation). Genotype was treated as fixed, rather than random, because the genotypes that we used

TABLE 1. MANOVA results performed on four subsets of reflectance and plasticity data for *Plantago lanceolata*. For the first three data sets, we show Wilks' lambda values for the effects of temperature treatment (Trt) and population (Pop) on reflectance pattern of preflowering and fruiting spikes over the spectral range of 370–850 nm. For the H + L data set, we show the effect of population on reflectance plasticity. Plasticity = $Ref_H - Ref_L$. Number of observations in each analysis: preflowering spikes, GH + L = 101, GH + H = 87, GH + M = 70, H + L = 37; fruiting spikes, GH + L = 79, GH + H = 66, H + L = 29. Temperature treatments: GH = Greenhouse, L = low, M = medium, H = high. Populations: NC = North Carolina, VA = Virginia, RI = Rhode Island. See Experiment 2, Methods for further explanation.

Treatments in data set		Source	Wilks' lambda	df	*F*	*P*
A) Preflowering spikes						
GH + L		Pop	0.2633	50	2.69	<0.0001
		Trt	0.2394	25	9.02	<0.0001
	Contrasts					
		Pop × Trt	0.4708	50	1.30	0.1190
		NC vs. RI	0.4956	25	2.89	0.0002
		NC vs. VA	0.5707	25	2.14	0.0068
		VA vs. RI	0.4727	25	3.17	<0.0001
GH + H		Pop	0.1982	50	2.84	<0.0001
		Trt	0.2081	25	8.68	<0.0001
		Pop × Trt	0.3213	50	1.74	0.0080
	Contrasts					
		NC vs. RI	0.5066	25	2.22	0.0066
		NC vs. VA	0.3556	25	4.13	<0.0001
		VA vs. RI	0.4609	25	2.67	0.0011
GH + M		Pop	0.1943	50	2.03	0.0023
		Trt	0.2503	25	4.79	<0.0001
		Pop × Trt	0.2246	50	1.78	0.0108
	Contrasts					
		NC vs. RI	0.3729	25	2.69	0.0025
		NC vs. VA	0.5353	25	1.39	0.1733
		VA vs. RI	0.4294	25	2.13	0.0162
H + L		Pop	0.0755	50	1.06	0.4644
B) Fruiting spikes						
GH + L		Pop	0.1841	50	2.61	<0.0001
		Trt	0.2159	25	7.12	<0.0001
		Pop × Trt	0.3673	50	1.27	0.1538
	Contrasts					
		NC vs. RI	0.4898	25	2.04	0.0163
		NC vs. VA	0.4687	25	2.22	0.0084
		VA vs. RI	0.3575	25	3.52	<0.0001
GH + H		Pop	0.1181	50	2.75	<0.0001
		Trt	0.1562	25	7.78	<0.0001
		Pop × Trt	0.3137	50	1.13	0.3125
	Contrasts					
		NC vs. RI	0.3487	25	2.69	0.0033
		NC vs. VA	0.3148	25	3.13	0.0009
		VA vs. RI	0.3620	25	2.54	0.0053
H + L		Pop	0.0025	50	1.53	0.3743

did not represent a random or haphazard sample of each population. We deliberately chose genotypes whose reflectance at low temperature spanned the range of reflectances observed in the low-temperature treatment of experiment 2. If a MANOVA showed that reflectance pattern from 370 to 850 nm differed among genotypes, we then performed one-way ANOVAs at every 10 nm from 370 to 850 nm to identify the wavelengths at which significant differences existed.

We also examined the correlations (SAS, 2000) among reflectance plasticity, high-temperature reflectance, and low-temperature reflectance to see if the differences in plasticity might best be explained by variation in reflectance at high temperature, variation at low temperature, or variation at both temperatures. These analyses were performed on spectral data at 550 nm and at 850 nm, in the visible and near-infrared regions, respectively, where plasticity was highest in experiment 2.

Results—The multivariate analyses showed that for both populations, genotypes significantly differed in reflectance pattern in each temperature regime (Fig. 7, Table 2). The NC genotypes differed much more at low temperature than at high temperature. At low temperature, NC genotypes differed significantly at every wavelength (Fig. 7A; $P < 0.00001$ in all ANOVAs). At high temperature, significant differences were restricted to 362–550 nm (ANOVAs: *P* value range: 0.0001–0.042) and 720–850 nm (ANOVAs: *P* value range: 0.0006–0.015). The RI population showed genotypic variation at both temperatures, but more at high temperature than did the NC population (Fig. 7B; low-temperature ANOVAs: *P* value range over the whole spectrum: 0.00000002–0.012). At high temperature, RI genotypes significantly differed in the visible and near-infrared regions: 400–620 nm, 700–820 nm (ANOVAs: *P* value range: 0.003–0.05 and 0.0092–0.046, respectively).

Genotypes significantly differed in reflectance plasticity in both populations (Fig. 7; Table 2). For the NC population, genotypic variation was significant at all wavelengths (ANOVAs: $P < 0.005$). For the RI population, significant genotypic variation was detected only in the regions of 510–610 nm and 710–850 nm (ANOVAs: *P* value range: 0.011–0.047 and 0.0051–0.042, respectively).

The correlations between reflectance plasticity, high-temperature reflectance, and low-temperature reflectance differed for the NC and RI populations. At 550 nm and for the NC population, plasticity was significantly negatively correlated with reflectance at low temperature (Fig. 7A; $r = -0.88$, $N = 26$,

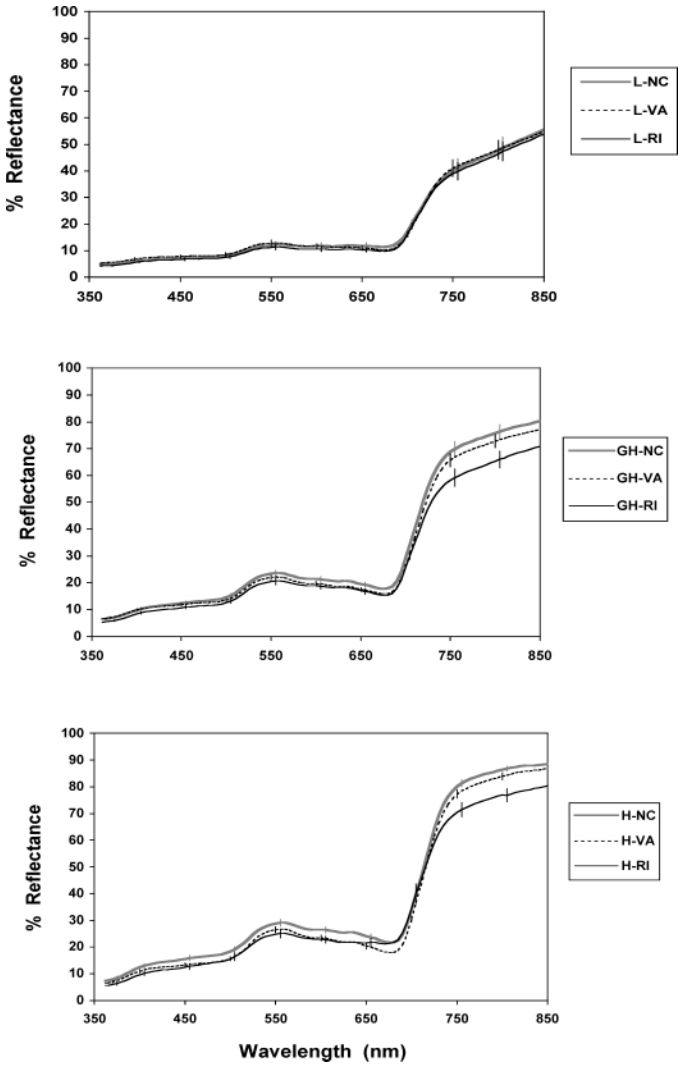

Fig. 4. Mean percentage reflectance of preflowering spikes of three populations of *Plantago lanceolata* shown by temperature treatment. See Fig. 3 for more explanation.

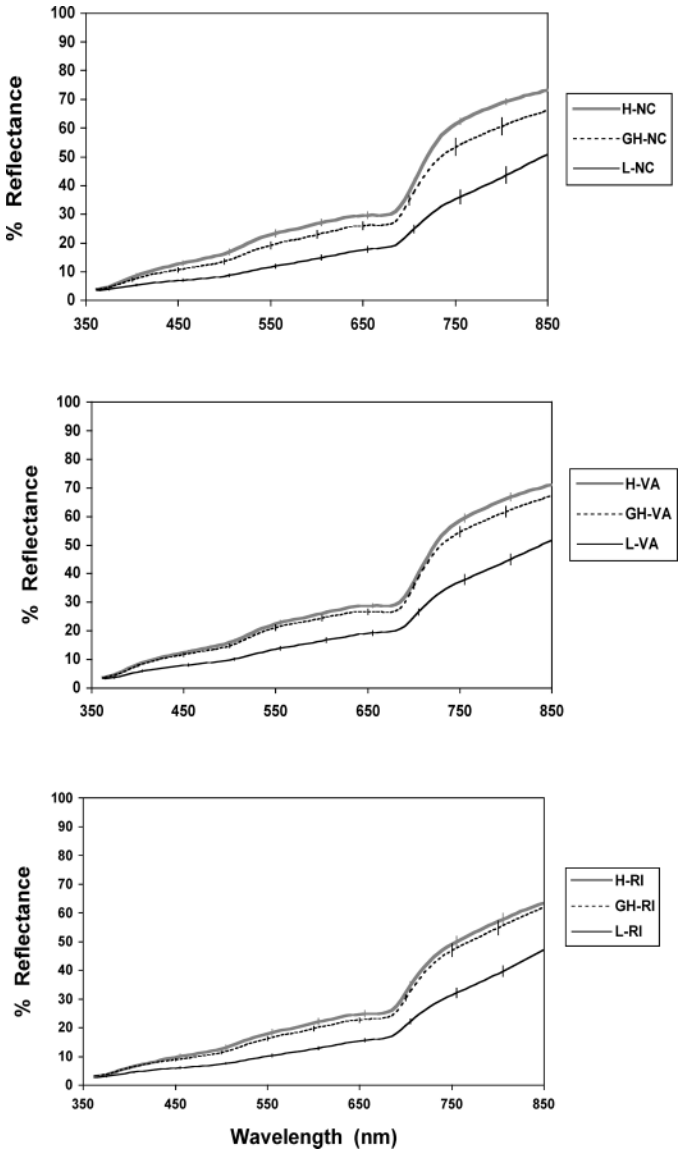

Fig. 5. Mean percentage reflectance of fruiting spikes of *Plantago lanceolata* for three temperature treatments shown by population. See Fig. 3 for more explanation.

$P < 0.0001$) but was not correlated with reflectance at high temperature ($r = 0.12$, $N = 26$, $P = 0.56$). The analysis at 850 nm showed the same pattern (correlation with low-temperature reflectance; $r = -0.98$, $N = 26$, $P < 0.0001$; with high-temperature reflectance: $r = -0.14$, $N = 26$, $P = 0.48$). Thus, all genotypes sampled reflected a lot at high temperature; only highly plastic genotypes reduced reflectance/darkened a lot at low temperature.

For the RI population, plasticity was correlated with reflectance at both low and high temperatures (Fig. 7B). At 550 nm, plasticity was negatively correlated with reflectance at low temperature but positively correlated with reflectance at high temperature (correlation with low temperature: $r = -0.44$, $N = 40$, $P = 0.004$; with high temperature: $r = 0.85$, $N = 40$, $P < 0.0001$). At 850 nm, however, only the correlation with low temperature reflectance was significant, and it was negative (low temperature: $r = -0.90$, $N = 40$, $P < 0.0001$; high temperature: $r = 0.07$, $N = 40$, $P = 0.68$). Thus, highly plastic genotypes not only reduced reflectance (darkened more) at low temperature, but also increased reflectance (lightened more) at high temperature, at least in the visible portion of the spectrum.

Experiment 4*—Methods*—The effect of temperature treatment on internal spike temperature was examined when preflowering spikes were placed outdoors in full sun. We used clones of 17 genotypes that had been used in the low-temperature and greenhouse treatments for experiment 2. On 1 of 3 days, we paired spikes from low-temperature (L) and greenhouse (GH) clones of the same genotype. A thermocouple wire was inserted into each spike to record the temperature (Temp) difference between the two spikes (Difference = Temp_L–Temp_{GH}). We were not able to record simultaneously the absolute temperature of both spikes.

To reduce the influence of other variables on our measurements, we placed clones of the same genotype side by side and always inserted thermocouple wires behind flowers facing the sun. Chosen flowers were positioned one-third the length of the spike from the distal end of the spike. Flowers completely covered the bare portion of the wires. All spikes were in their natural vertical position at the time of measurement. Measured spikes were of similar height from the ground (ground to spike bottom: range = 15–40 cm; difference between paired spikes: mean ± 1 SE = 1.19 ± 0.21 cm) and of similar length (distance from spike top to bottom: range = 1.1–2.3 cm; difference between paired spikes: mean ± 1 SE = 0.14 ± 0.03 cm). Our measurements were made when no wind could be detected by our sense of touch and when there was a slight breeze. To approach the temperature differences in the

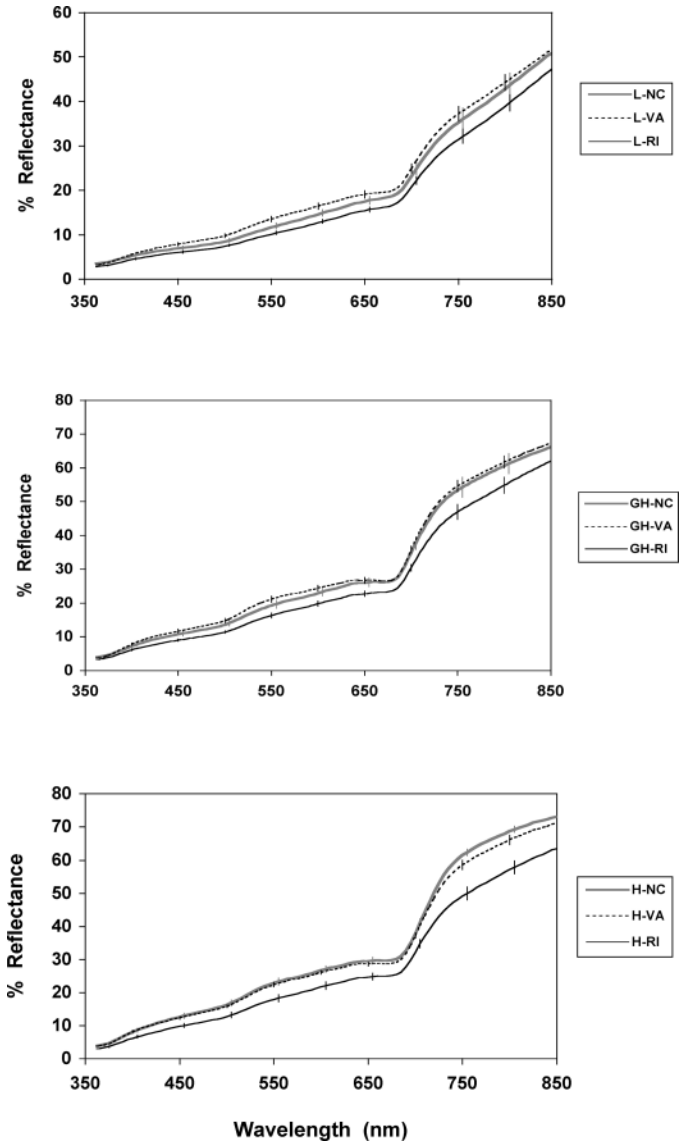

Fig. 6. Mean percentage reflectance of fruiting spikes for three populations of *Plantago lanceolata* shown by temperature treatment. See Fig. 3 for more explanation.

absence of wind, we recorded the highest temperature difference that we could detect over 2–3 min of ''relative calm.'' We also recorded how far below zero the temperature difference fell.

Results—Spikes from the low-temperature treatment were always hotter than spikes from the greenhouse treatment. Maximal differences ranged from 0.2° to 2.6°C (Fig. 8). As one might expect, the temperature differences between spikes declined as wind velocity rose and increased as velocity decreased. No difference ever fell below zero, although the difference dropped to zero for some pairs of spikes.

DISCUSSION

Characterization of reflectance plasticity—The data show that for *Plantago lanceolata*, spike reflectance and color are temperature-sensitive. This is not to say that an individual spike changes reflectance/color with short-term, e.g., diurnal temperature change. We saw no evidence of that. Rather, spike

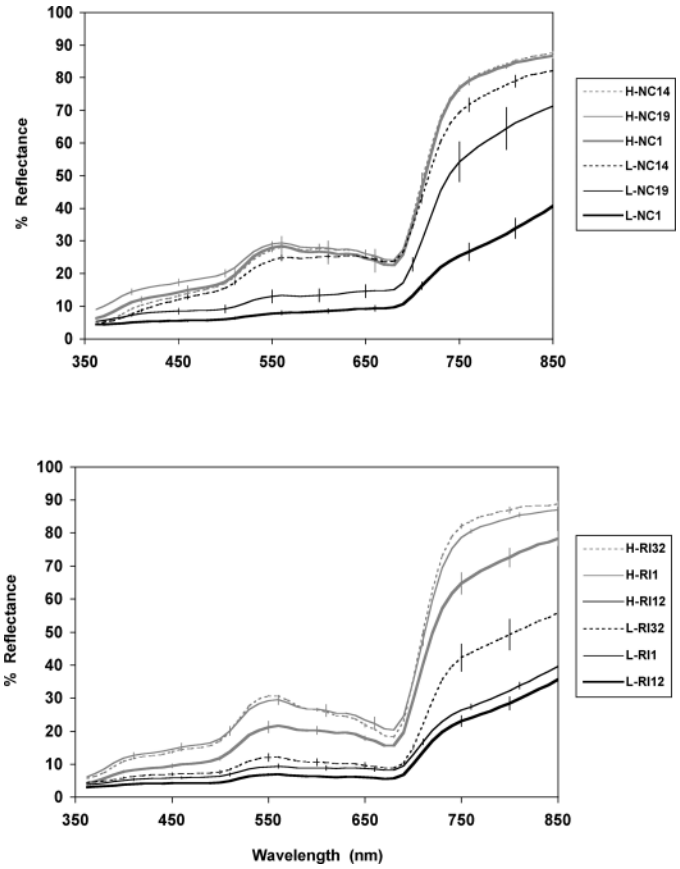

Fig. 7. Mean percentage reflectance of three representative NC genotypes (1, 14, 19) and three RI genotypes (1, 12, 32) in high (H) and low (L) temperature treatments.

reflectance appears to be determined by the ambient temperature at the time of spike development. When ambient temperature changes, a flowering plant produces new spikes having a different reflectance/color. Thus, temperature change during the flowering season can produce seasonal changes in reflectance in natural populations, which we observed in experiment 1. Although we did not measure leaf reflectance,

TABLE 2. Results of MANOVA tests to measure the effect of genotype on reflectance pattern and plasticity from 370 to 850 nm in North Carolina (NC) and Rhode Island (RI) populations of *Plantago lanceolata*. Populations were analyzed separately. Shown are Wilks' lambda values for the within-population genotypic effects on reflectance of preflowering spikes at high (H) and low (L) temperatures and on reflectance plasticity. Plasticity = $Ref_H - Ref_L$. Number of genotypes used in each analysis: NC = 9, RI = 14.

Population	Wilks' lambda	df	*F*	*P*
A) High temperature				
NC	0.00000295	104	2.34	0.0009
RI	0.00000770	169	2.18	<0.0001
B) Low temperature				
NC	0.00000004	104	5.44	<0.0001
RI	0.00000056	169	3.58	<0.0001
C) Plasticity				
NC	0.00000012	104	4.00	<0.0001
RI	0.00000644	169	2.09	<0.0001

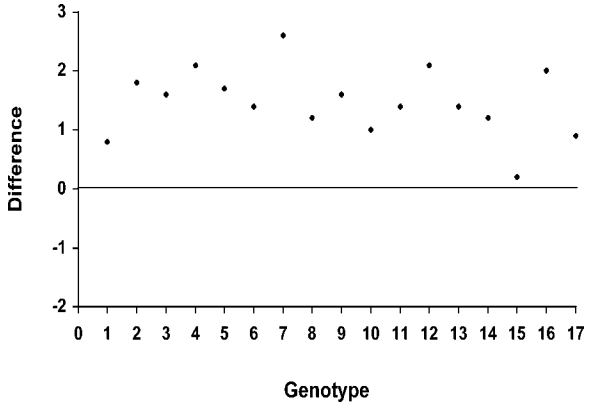

Fig. 8. Maximal differences (°C) in spike temperature ($Temp_L - Temp_{GH}$) between paired preflowering spikes for 17 genotypes grown at low temperature and in the greenhouse. See experiment 4 for more explanation.

we observed no temperature-associated color changes in the leaves of the experimental plants (E. Lacey, personal observation).

Temperature affects reflectance most in the visible (525–650 nm) and near-infrared (725–850 nm) regions. The near-infrared region shows greater sensitivity on an absolute scale, but the visible region shows greater sensitivity on a relative scale. As evidenced by experiment 2, temperature-induced changes are reversible and are not a consequence of the developmental stage of the clone. When temperature was increased in the growth chamber, spike reflectance increased; when temperature was subsequently lowered, reflectance decreased. This suggests that the seasonal changes that one sees in natural populations are reversible rather than developmentally canalized or determined by declining resources. In NC, reflectance, on average, increases through the reproductive season. In Orsay, France, spike color, on average, lightens from spring to summer and darkens in autumn (E. Lacey, personal observation).

These plastic responses can be measured in days. Although we measured reflectance 2 wk after we changed the ambient temperature in experiments 2 and 3, we noticed that spikes appearing 1 wk after each temperature change had already changed in color from those that had been produced under the previous temperature. Thus, changes can occur fairly quickly on a seasonal time scale.

The reflectance pattern of a single spike changes during ontogeny. As demonstrated by the results of experiment 2, temperature sensitivity extends from the preflowering stage into the period of fruit and seed development on a spike. However, the effects of temperature in the visible and near-infrared regions diminish. The observed lack of a strong correlation between reflectance in the preflowering and fruiting stages suggests that there is a change in the relative contributions of different floral structures to reflectance associated with ontogeny. Prior to flowering, reflectance in the visible portion of the spectrum appears to be associated with the variable deposition of pigments in the bracts that cover the flowers (E. Lacey, personal observation). During flowering and fruiting, petal lobes may increasingly influence reflectance. Pigment deposition also varies in the petals.

One might ask whether differences in light between the greenhouse and growth chamber could have explained our results for experiment 2. This is not likely. First, we saw strong and comparable changes in reflectance in the growth chamber alone by changing only temperature. Also, in a preliminary experiment, shaded clones of experimental genotypes did not produce noticeably darker spikes, or bracts, than did nonshaded clones (E. Lacey, personal observation).

Reflectance and plasticity in reflectance, i.e., degree of temperature sensitivity, are likely to be at least partially genetically controlled. This is suggested by the significant population differences detected in experiment 2 and by the significant genotypic differences detected within both NC and RI populations in experiment 3. Also in experiment 3, genotypes differed in plasticity. In the NC population, the more plastic genotypes lowered reflectance (darkened) more at low temperature than did less plastic genotypes. In the RI population, the more plastic genotypes both lowered reflectance (darkened) more at low temperature and increased reflectance (lightened) more at high temperature. One could argue that the population differences in reflectance could have been explained by the difference in environments of the populations from which sample genotypes were initially drawn for experiment 2, and we cannot dismiss this possibility. It is harder, however, to use this counter-argument for experiment 3. Plants used in experiment 3 had gone through two ''clone-generations'' and been grown in the same environment for 1.5 yr before conducting the experiment. Thus, it is likely that mean spike reflectance and plasticity in reflectance have at least a partial genetic basis and, therefore, the potential to evolve.

The data from our final experiment indicate that spike reflectance influences internal flower temperature and that reflectance plasticity is a mechanism by which plants can partially thermoregulate their reproduction and the embryonic development of their offspring. In spite of the technical difficulties of measuring temperature outdoors, we did observe that sunlight warmed low-temperature (darker-colored) clones more than it did greenhouse (lighter-colored) clones in little to no wind in experiment 4. When breezes arose, the temperature differences declined, but they never reversed themselves. Darker bodies generally absorb more solar radiation, and this likely explains our results. *Plantago lanceolata* spikes are cylindrical and opaque, and, therefore, light that is not reflected is mostly absorbed. Because reflectance differences persist into the fruiting phase, reflectance probably also influences spike temperature during fruit development. How much reflectance may alter the internal temperature of spikes in natural populations and over a longer time period is currently unclear. Current measurements of internal spike temperature in the field will provide us with this information.

Possible evolutionary significance of reflectance plasticity—Postzygotic temperature (temperature during parental flowering and early offspring development of offspring on the maternal parent) can influence offspring fitness in *P. lanceolata*. Lacey and Herr (2000) observed that temperatures resembling mean monthly temperature for July, late in the flowering season in the North Carolina piedmont, produced offspring that were almost 50% more fit than did temperatures resembling mean monthly temperature for May, earlier in the flowering season. In light of this previous study, the phenotypic plasticity in spike reflectance (thermoregulatory capability) could be adaptive. When the reproductive season is cool, as in early spring, if parents lower spike reflectance (darken), then they should increase internal floral and fruit temperature to a more optimal level from the point of view of offspring

fitness. Selection for such thermoregulation may be strong. Seed predation increases greatly throughout the flowering season in the NC piedmont (Lacey et al., 2003). Thus, although later-flowering plants appear to produce offspring of higher quality because of the warmer temperatures at that time, total seed production suffers. Temperature-sensitive plasticity in spike reflectance would offset the negative effect of earlier flowering on offspring fitness and allow plants to produce many higher-quality seeds. If this hypothesis correct, then reflectance plasticity would be the first example of a trait that produces adaptive parental effects in a plant species. The hypothesis is currently being tested more directly.

Reflectance plasticity illustrates where we might generally look for and find traits producing adaptive parental effects in plants. Maternal effects were identified first by quantitative geneticists and defined as the effects of the environment provided by the mother on the growth and development of her offspring (Dickerson, 1947; Willham, 1963; Cheverud, 1984; Cheverud and Moore, 1994). This definition can be extended to include fathers to provide a definition for parental effects. Parental effects, defined in this way, differ from ''parental (or maternal) environmental effects'' and ''environmentally induced parental effects,'' which have been the focus of many plant studies. In such studies, researchers have deliberately manipulated the parental environment to measure the phenotypic responses in offspring (e.g., see reviews: Roach and Wulff, 1987; Gutterman, 1992; Schmitt et al., 1992; Wulff, 1995; Donohue and Schmitt, 1998; Lacey, 1998; Shaw and Byers, 1998). Such studies almost always detect parental environmental effects, e.g., postzygotic temperature effects in *P. lanceolata* (Lacey, 1996; Lacey and Herr, 2000).

What is missing from these plant studies is any indication of how these parental environmental effects might influence the evolution of natural plant populations, i.e., evidence that these effects are evolutionarily important. If these effects are important, then there should exist traits whose evolution is being or has been influenced by the effects. Because reproductive traits can influence offspring phenotype and fitness (e.g., Lacey and Pace, 1983; Case et al., 1996; Ollerton and Diaz, 1999; Picó and Retana, 2000; Wolfe and Burns, 2001; Galloway, 2002; Lacey et al., 2003), reproductive traits are likely to evolve in response to parental environmental effects. If a parental environment fluctuates such that an environmental state often reduces offspring fitness via a parental environmental effect, then selection should favor the evolution of a parental reproductive trait that counteracts the deleterious environmental effect. If the parental trait manipulates offspring environment to counteract the effect and is energetically costly, then we would have evidence for adaptive parental effects, using the quantitative genetics definition, and also for parental care (Fig. 1; Clutton-Brock, 1991; Cheverud and Moore, 1994; Alcock, 2001). Spike reflectance (thermoregulatory capability) is a possible example of this. It may be analogous to animal behaviors that regulate the temperature of developing offspring, e.g., birds sitting on their eggs. Also, reflectance plasticity may be energetically costly because of pigments that appear in floral structures at low temperature. A second example of a reproductive trait that may produce adaptive parental effects and that may represent parental care is heliotropism, or solar tracking, of flowers in some alpine species (e.g., Galen and Stanton, 2003). In both cases, deleterious parental temperature effects, i.e., low temperature, may have selected for the ability to thermoregulate the reproductive structures.

It is presently unclear whether or not *P. lanceolata* is unique in having the ability to alter the reflectance of its reproductive structures in response to temperature. We suspect not, however, for several reasons. First, reflectance is an ecologically important property of a plant because it influences metabolic rate. Plants can reduce cellular and molecular stress induced by intense heat by increasing leaf reflectance and increasing the level of pigments that can dissipate high thermal energy (e.g., Ehleringer and Bjorkman, 1978; Gates, 1980; Demmig-Adams and Adams, 1992; Tattini et al., 2000). Alternatively, plants can reduce cold-induced stress by decreasing leaf reflectance and increasing the level of pigments that absorb solar energy, which increases internal plant temperature (e.g., Krog, 1955; Gates, 1980). Second, color is known to influence temperature within the reproductive unit in several other species. Purple cones have higher internal temperatures then do green cones in *Abies concolor* (Sturgeon and Mitton, 1980). Warmer floral temperatures are associated with darker flowers in *Lotus corniculatus* and *Papaver radicatum* (Mølgaard, 1989; Jewell et al., 1994). Third, color of reproductive structures can vary geographically. The 19th century botanists Bonnier (1888) and Kerner (1894) reported that spikes of widely distributed grasses, sedges, and rushes darken with increasing altitude and that flowers are often more strongly pigmented at high altitudes. Our observations are consistent with this pattern. The RI and NC populations of *P. lanceolata* that we sampled were the least and most reflective populations, respectively. Also, they differed in pattern of plasticity. In *Abies concolor*, purple-coned individuals increase in frequency with increasing elevation relative to green-coned individuals (Sturgeon and Mitton, 1980). With increasing latitude or altitude, darker colors/reduced reflectance may improve seed production and offspring fitness. Studies of melanism (e.g., reviewed by Majerus, 1998) indicate that the frequency of melanics in populations of many animal species is generally positively correlated with latitude and that this latitudinal variation reflects past evolutionary responses to differences in local or regional climate. Parallel evolutionary processes may be at work on the reflectance of reproductive structures in plants.

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