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Two reproductive traits show contrasting genetic architectures in *Plantago lanceolata*

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Abstract

In many species, temperature-sensitive phenotypic plasticity (i.e., an individual's phenotypic response to temperature) displays a positive correlation with latitude, a pattern presumed to reflect local adaptation. This geographical pattern raises two general questions: (a) Do a few large-effect genes contribute to latitudinal variation in a trait? (b) Is the thermal plasticity of different traits regulated pleiotropically? To address the questions, we crossed individuals of *Plantago lanceolata* derived from northern and southern European populations. Individuals naturally exhibited high and low thermal plasticity in floral reflectance and flowering time. We grew parents and offspring in controlled cool- and warm-temperature environments, mimicking what plants would encounter in nature. We obtained genetic markers via genotype-by-sequencing, produced the first recombination map for this ecologically important nonmodel species, and performed quantitative trait locus (QTL) mapping of thermal plasticity and single-environment values for both traits. We identified a large-effect QTL that largely explained the reflectance plasticity differences between northern and southern populations. We identified multiple smaller-effect QTLs affecting aspects of flowering time, one of which affected flowering time plasticity. The results indicate that the genetic architecture of thermal plasticity in flowering is more complex than for reflectance. One flowering time QTL showed strong cytonuclear interactions under cool temperatures. Reflectance and flowering plasticity QTLs did not colocalize, suggesting little pleiotropic genetic control and freedom for independent trait evolution. Such genetic information about the architecture of plasticity is environmentally important because it informs us about the potential for plasticity to offset negative effects of climate change.

KEYWORDS

adaptive plasticity, genetic architecture, geographical cline, linkage map, phenotypic plasticity, QTL mapping, thermal plasticity

1 | INTRODUCTION

Researchers have posited two mechanisms that might allow populations to persist in situ, given ongoing climate change: phenotypic plasticity and local adaptation (Anderson, Inouye, McKinney, Colautti, & Mitchell-Olds, 2012; Bradshaw & Holzapfel, 2008; Chevin, Lande, & Mace, 2010; Davis, Shaw, & Etterson, 2005; Jump & Penuelas, 2005; Ravenscroft, Whitlock, & Fridley, 2015). Phenotypic plasticity is the ability of an individual to modify its phenotype in response to environmental change. If individuals in a species can modify their phenotypes in ways that improve fitness under rising temperatures, and/

or changes in precipitation patterns, then these populations should increase their probability of local persistence. Local adaptation refers to genetic change in a population that improves average fitness (including survival and reproduction) over generations in response to local selective pressures. However, adaptation occurs slowly, over generations, in a population. If adaptation can occur more quickly than the rate of climate change, then populations have an increased probability of survival. Phenotypic plasticity might facilitate this adaptation via the Baldwin effect (Simpson, 1953) by expanding the range of phenotypes expressed in a population, which would allow selective forces to more easily discriminate among genotypes, thus speeding adaptive evolution (Auld, Agrawal, & Relyea, 2009; Crispo, 2007; Kitano, 2004; Lande, 2009; Schlichting, 2008; Vedder, Bouwhuis, & Sheldon, 2013).

Thus far, empirical studies that allow us to evaluate the relative contributions of either mechanism to population persistence are rare. One limitation is that we know little about the genetic architecture of traits locally adapted to different climates. This includes the genetics of phenotypic plasticity in these traits (El-Soda, Malosetti, Zwaan, Koornneef, & Aarts, 2014; Lee, Gould, & Stinchcombe, 2014; Nicotra et al., 2010; Yadav, Dhole, & Sinha, 2016). Genetic architecture is important because it informs us about the extent to which individual loci affect trait mean values, demonstrate plasticity and have pleiotropic effects on multiple traits. These factors have far-reaching implications for the potential for natural selection on traits and their plasticities to offset short-term negative effects of environmental change and influence the potential for adaptive shifts (Lee et al., 2014; Martin & Orgogozo, 2013).

Because temperature is a major determinant of climate, there is a particular need to understand the genetic architecture of traits that are temperature-sensitive. Many species, particularly ectotherms (i.e., most species on Earth), exhibit thermal plasticity (temperature-sensitive phenotypic plasticity) in traits contributing to fitness. For ectotherms, which rely on external heat sources to mediate internal body temperature, temperature-sensitive plasticity allows organisms to acclimatize to a new temperature through adjustments in: (a) behaviour, such as movements through microhabitats and solar tracking (Clench, 1966; Ehleringer & Forseth, 1980; Huey, 1991; Kudo, 1995; Webster & Weathers, 1990); (b) phenology of sensitive life stages, such as bud, flower and fruit emergence, and laying/birthing date in animals (Crick, Dudley, Glue, & Thomson, 1997; Fitter & Fitter, 2002; Visser & Holleman, 2001); and (c) cellular physiology, such as of cellular membranes and gene expression (Angilletta, 2009; Hazel, 1995; Huey & Bennett, 1990; Huey & Stevenson, 1979; Lacey & Herr, 2005; Marmur & Doty, 1962; Somero, 1995).

Ectotherms generally show positive correlations between their thermal plasticity (including thermal "tolerance" and "acclimation") and latitude and altitude (Angilletta, 2009; Ghalambor, Huey, Martin, Tewksbury, & Wang, 2006; Hoffmann, Sørensen, & Loeschcke, 2003; Liefting, Hoffmann, & Ellers, 2009). For example, latitudinal/altitudinal variation has been observed in frog developmental rate (Laugen, Laurila, Räsänen, & Merilä, 2003), thermal tolerance in insects (Addo-Bediako, Chown, & Gaston, 2000; Gaston & Chown, 1999) and in lizards (van Berkum, 1988), leaf shape in trees (Royer, Meyerson, Robertson, & Adams, 2009), and floral colour in herbaceous species (Anderson, Lovin, Richter, & Lacey, 2013; Lacey, Lovin, Richter, & Herington, 2010). These geographical patterns provide unique opportunities to clarify the genetic architecture of traits that are sensitive to temperature and to explore the genetic basis of evolutionary divergence in plasticity among populations within a species. Presently such information is rare (Ågren, Oakley, Lundemo, & Schemske, 2017; Alonso-Blanco & Méndez-Vigo, 2014; Alonso-Blanco, Mendez-Vigo, & Koornneef, 2004; Anderson, Lee, Rushworth, Colautti, & Mitchell-Olds, 2013; Anderson, Willis, & Mitchell-Olds, 2011; Des Marais, Hernandez, & Juenger, 2013; Dittmar, Oakley, Conner, Gould, & Schemske, 2016; Gerken, Eller, Hahn, & Morgan, 2015; Hall, Basten, & Willis, 2006; Leinonen et al., 2013; Remington, 2015).

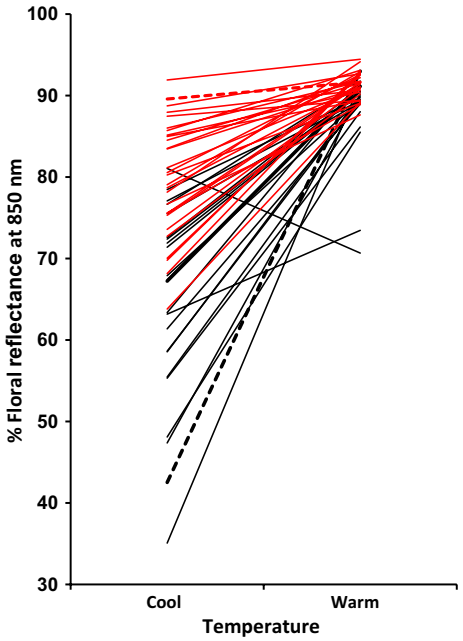

FIGURE 1 Reaction norms for *Plantago lanceolata* genotypes from Veno, Denmark, (black) and Hameau de St. Felix, France (red), grown under cool (15°C day/10°C night) and warm (27°C day/20°C night) temperature. Dashed lines show reaction norms of F_0 parents from Denmark and France that were used in this study

The natural geographical variation in thermal plasticity of floral reflectance in *Plantago lanceolata*, a widespread, perennial herb, allowed us to explore the natural variation in genetic architecture along a latitudinal gradient. *Plantago lanceolata* individuals vary in their ability to respond to the ambient temperature during flower development. As ambient temperature changes throughout the lengthy flowering season, a thermally plastic individual modifies the colour/reflectance of its newly developed flowers. This sensitivity is strong in two regions of the electromagnetic spectrum, the visible and NIR (near infrared) regions (Lacey & Herr, 2005). Plastic individuals produce darker, less reflective flowers in cool temperatures and lighter, more reflective flowers in warm temperatures—e.g., typically, dark flowers in spring and autumn and light flowers in

summer (Lacey & Herr, 2005). Results of multiple experiments are consistent with the hypothesis that this thermal plasticity is locally adaptive (Lacey, Lovin, & Richter, 2012; Lacey et al., 2010; Marshall, Batten, Remington, & Lacey, 2019). However, not all individuals are thermally plastic. Nonplastic individuals produce lightly coloured/highly reflective flowers, regardless of ambient temperature (Lacey & Herr, 2005). Temperature-sensitive plasticity is positively correlated with latitude and altitude in the species' native European range (Figure 1 and Lacey et al., 2010), and this geographical pattern is better explained by local adaptation than by neutral evolutionary factors (Marshall et al., 2019).

In this study, we used the latitudinal variation in *P. lanceolata* to explore the genetic architecture of thermal plasticity in floral reflectance and to assess the architectural difference(s) between northern and southern European populations. We produced an F_2 mapping family from two crosses of parental genotypes derived from northern (Sweden and Denmark) and southern (France and Italy) populations. Northern and southern parents genetically differed not only in floral reflectance, but as we discovered after we had selected our parental genotypes, also in time of flowering onset, which we refer to as flowering time. Therefore, we collected phenotypic data for both traits from parental, F_1 and F_2 generations grown in controlled cool and warm thermal environments. These temperatures simulated those that plants encounter in their natural habitats during the reproductive season. Then we looked for quantitative trait loci (QTLs) associated with both traits.

Our data provided an opportunity to address two general evolutionary questions about the genetic architecture of locally adapted traits. First, to what extent do large-effect genes contribute to geographical variation in locally adapted quantitative traits? The Fisherian polygenic model of inheritance (Fisher, 1930, 1919) proposes that genetic differences between populations are explained by allelic variation in many genes, each having a small effect on the phenotype of the adapted trait. Thus, geographical variation would probably have arisen over time from many small mutations in multiple genes. The alternative model predicts that much of the genetic difference between locally adapted populations can arise from allelic variation in one or just a few large-effect genes (Orr, 1998, 2005; Robertson, 1967). Numerous recent empirical studies have been cited in support of each model (Martin & Orgogozo, 2013; Remington, 2015; Rockman, 2012; Wellenreuther & Hansson, 2016), and neither model is likely to represent a universal principle of genetic architecture (Dittmar et al., 2016; Remington, 2015). Factors that may favour variation involving large-effect alleles include traits that are under selection for different optima in different environments (Dittmar et al., 2016; Remington, 2015), traits with a small number of available genetic mechanisms to produce a particular adaptive change (Frankel, Wang, & Stern, 2012; Lee et al., 2014; McGregor et al., 2007), and moderate rates of gene flow (Yeaman & Whitlock, 2011). In our study, we have evidence that reflectance plasticity is under selection for different optima along a latitudinal gradient as described above. Differentiation in flowering time could also involve local adaptation, as has been reported in other species (Anderson, Lee, et al., 2013; Dittmar, Oakley, Ågren, & Schemske, 2014; Leinonen et al., 2013).

Second, is thermal plasticity variation in multiple locally adapted traits regulated pleiotropically? If environmental sensitivity to a stimulus shares a common molecular mechanism (i.e., a genetic pathway or physiology important for sensing and responding to thermal fluctuations), then we may find that the plastic responses of vastly different phenotypes are under pleiotropic genetic control. Pleiotropy can either constrain or facilitate adaptive evolution depending on whether the pleiotropic effects of a gene are antagonistic or synergistic for fitness (Lee et al., 2014; Martin & Orgogozo, 2013). It has been proposed that genes with pleiotropic regulatory effects that are concordant with complex adaptations may represent "hotspot" genes involved in convergent evolution of adaptive traits (Martin & Orgogozo, 2013; McGregor et al., 2007). However, empirical data to test this are rare. QTL studies in *Arabidopsis thaliana* (Méndez-Vigo et al., 2016) and *A. lyrata* (Quilot-Turion et al., 2013) have observed colocalization of loci underlying temperature-sensitive plasticity of flowering time and vegetative traits. Several molecular studies have suggested that the photoreceptor gene *PHYB* helps to thermoregulate plasticity in both plant flowering time and anthocyanin accumulation (Gu, Wang, Hu, & Hao, 2019; Li, Mao, et al., 2012b; Zhou et al., 2019). However, other studies suggest that anthocyanin accumulation is thermoregulated by pathways independent of flowering time (Gu et al., 2019; Zhang et al., 2017, 2016).

To address the above evolutionary questions with *P. lanceolata*, we developed a de novo genetic recombination map from markers attained via double-digest restriction associated digest sequencing (ddRADseq). Then we mapped QTLs associated with floral reflectance and flowering time under warm and cool conditions in order to characterize the genetic architecture underlying the evolutionary divergence of *P. lanceolata* populations in these traits and in their plasticities. The specific questions that we addressed were: (a) How much of the geographical differences for reflectance and flowering and their plasticities are explained by large-effect QTLs? (b) Do plasticity QTLs colocalize with single-environment QTLs? Because phenotypic differences in plasticity involve differential responses to cool temperature (Lacey et al., 2010), we predicted that the plasticity QTLs would colocalize only with the cool-environment QTLs. Also, we expected to find a strong additive contribution of northern QTL alleles to high plasticity, at least for reflectance. (c) Do the QTLs for plasticity in floral reflectance and flowering time colocalize? Colocalizing QTLs would be consistent with pleiotropic control of plasticity in the two traits, although independent control by separate closely linked genes could not be ruled out.

2 | METHODS

2.1 | Biology of *Plantago lanceolata*

Plantago lanceolata L. (English, or ribwort, plantain), Plantaginaceae, is a temperate, weedy, herbaceous perennial, native to Eurasia but

now well established in disturbed areas, lawns and grasslands in North America (Cavers, Bassett, & Crompton, 1980) and in other continents. Under short-day conditions, *P. lanceolata* grows vegetatively as a rosette. Under long days, the species produces flowers in compact inflorescences, called spikes, at the ends of leafless scapes. A plant may produce spikes throughout the reproductive season, which in some regions can last for 6 months. Flowers are protogynous, gynodioeceous, self-incompatible (Ross, 1973; Van Damme, 1983) and predominately wind-pollinated (Cavers et al., 1980). Many traits show intragenerational and intergenerational plasticity (Bradshaw, 1965; Case, Lacey, & Hopkins, 1996; Lacey & Herr, 2005, 2000; Primack & Antonovics, 1981; Schmitt, Niles, & Wulff, 1992; van Tienderen & van der Toorn, 1991a, 1991b; Van Hinsberg & Van Tienderen, 1997; Van Tienderen, 1990, 1992; Wolff, 1987; Wolff & Van Delden, 1987). Most *P. lanceolata* individuals exhibit temperature-sensitivity in floral colour and, more broadly, reflectance. In response to the natural change in ambient temperature during a reproductive season, plastic individuals produce new flowers of different colour/reflectance, based on the ambient temperature at the time of flower production (Lacey & Herr, 2005; Stiles, Cech, Dee, & Lacey, 2007). However, the degree of plasticity varies from highly plastic (e.g., a difference in percentage of light reflected at 850 nm of ~40% between spikes developed at warm vs. cool temperature) to nonplastic (Lacey & Herr, 2005; Lacey et al., 2010). Nonplastic individuals produce lightly coloured/highly reflective flowers, regardless of ambient temperature. Nonplastic individuals that constitutively produce dark/ poorly reflective flowers have rarely been found (Lacey et al., 2010).

Thermal plasticity in floral reflectance is positively correlated with latitude and altitude, and analyses suggest that these correlations reflect local adaptation to the duration of the reproductive season and the amount of time during the reproductive season when temperatures are low (Lacey et al., 2010; Marshall et al., 2019). Experiments indicate that a plant that can warm reproductive tissues during cool periods of the reproductive season increases its seed production during that period, without a fitness cost during warm periods (Lacey et al., 2012). Darker, less reflective flowers absorb more incoming solar radiation than lighter, more reflective flowers, thereby helping to warm reproductive tissues, which can improve seed production and offspring fitness (Lacey, 1996; Lacey & Herr, 2000, 2005). Additionally, population pairwise comparisons of neutral genetic differentiation, phenotypic differentiation and environmental differences, along with gene flow data, together indicate that natural selection best explains the evolutionary divergence in plasticity along latitudinal and altitudinal gradients (Marshall et al., 2019).

Flowering onset and end times are photoperiodically controlled in *P. lanceolata* (Snyder, 1948), but warm temperatures accelerate spike and floral development (Case et al., 1996; Lacey, 1996). Little is known about the geographical variation in flowering time; however, flowering phenology and climatic gradients strongly covary in other perennial species (Dittmar et al., 2014; García-Gil, Mikkonen, & Savolainen, 2003; Hall & Willis, 2007; Lacey, 1988; Leinonen et al., 2013; Olsson & Ågren, 2002; Waldmann, Garcia-Gil, & Sillanpää, 2005). The genetic architecture of flowering time in model species includes many temperature- and photoperiod-sensitive genes. Only a few, however, have been shown to contribute to natural geographical variation in flowering time (e.g. Brachi et al., 2010; Burgarella et al., 2016; Dittmar et al., 2014; Grillo, Li, Hammond, Wang, & Schemske, 2013).

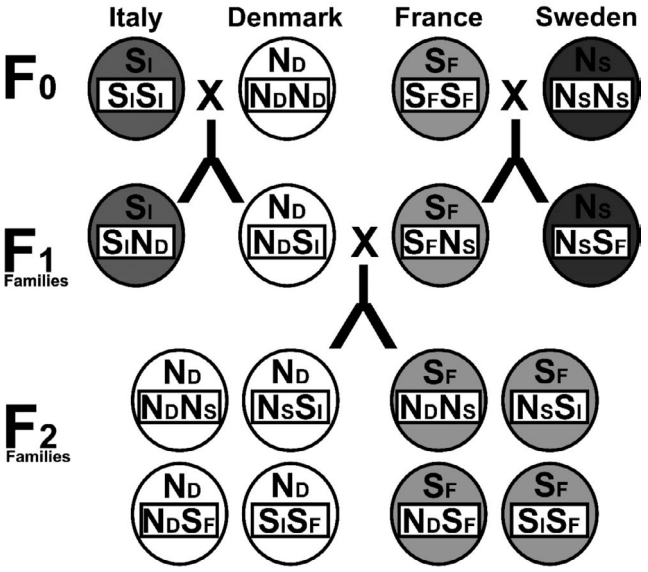

FIGURE 2 Diagram of reciprocal out-crossing design for creation of F_2 mapping family. Outer circles (cytoplasmic DNA), inner rectangles (nuclear DNA). Alleles designate inheritance from F_0 parent. Nuclear alleles in F_2s can be homozygous for northern- or southern-derived alleles, or heterozygous

2.2 | Crossing design

In 2012, because the species is an obligate outcrosser, we reciprocally outcrossed two northern genotypes from Veno, Denmark (56.55°N, 8.63°E), and Uppsala, Sweden (59.94°N, 17.39°E), with two southern genotypes from Aprilia, Italy (41.6°N, 12.65°E), and Hameau de St. Felix, France (43.58°N, 3.97°E), to produce an F_2 mapping population (Figure 2; Table 1). We selected genotypes from different European populations to minimize the chances of self-incompatibilities in later generations and provide greater opportunity to evaluate the degree of genetic heterogeneity between populations from similar latitudes. The northern and southern parents displayed high and low thermal plasticity of floral reflectance (Figures 3 and 4), and represented plasticity extremes found in a sample of 29 European *P. lanceolata* populations (e.g., Figure 1; Lacey et al., 2010). Parents were progeny of genotypes that had been collected from wild populations in 2000 and induced to produce offspring (i.e., the parents in this study) in a common environment in order to reduce maternal environmental effects (see details in Lacey et al., 2010). The reciprocal crosses (Danish × Italian and Swedish × French) yielded two hybrid F_1 families with reciprocal (northern and southern) cytoplasms (Figure 2). In

TABLE 1 Phenotypic means ± 1 *SE* of floral reflectance and flowering time for F_0 and F_1 parental genotypes, and for the F_2 mapping population

Genotype (Generation)	Temp.	Sweden (F_0)	Denmark (F_0)	France (F_0)	Italy (F_0)	Denmark × Italy (F_1)	France × Sweden (F_1)	All (F_2)	Danish cytoplasm (F_2)	French cytoplasm (F_2)
% Floral reflectance	Cool	59.41 ± 3.02 (4)	55.65 ± 4.43 (5)	85.98 ± 0.85 (6)	78.45 ± 2.45 (6)	70.86 ± 3.06 (8)	62.29 ± 2.93 (6)	68.28 ± 0.61 (481)	67.94 ± 0.84 (255)	68.67 ± 0.87 (226)
	Warm	91.01 ± 1.05 (2)	91.25 ± 0.73 (2)	91.77 ± 0.40 (5)	92.41 ± 0.60 (5)	92.69 ± 0.93 (7)	90.15 ± 0.92 (5)	90.98 ± 0.09 (478)	90.91 ± 0.12 (251)	91.06 ± 0.13 (227)
	Plasticity	*31.60 35%*	*35.60 39%*	*5.79 6%*	*13.96 15%*	*21.83 24%*	*27.86 31%*	22.96 ± 0.61 (453) *25%*	23.14 ± 0.86 (240) *25%*	22.75 ± 0.87 (213) *25%*
Flowering time (days)	Cool	90 ± 13 (4)	69 ± 9 (8)	34 ± 6 (6)	30 ± 2 (5)	45 ± 7 (7)	76 ± 11 (6)	73 ± 1 (484)	72 ± 1 (257)	73 ± 1 (227)
	Warm	89 ± 10 (2)	21 ± 4 (2)	16 ± 1 (5)	15 ± 1 (4)	16 ± 1 (6)	19 ± 1 (5)	24 ± 1 (475)	24 ± 1 (252)	25 ± 1 (223)
	Plasticity	*−1 2%*	*−48 230%*	*−18 116%*	*−15 102%*	*−29 181%*	*−57 300%*	−48 ± 1 (453) *200%*	−48 ± 1 (239) *200%*	−48 ± 1 (214) *192%*

Note: The number of F_0 and F_1 parent clones and F_2 genotypes used to estimate values are given parenthetically. Mean values were estimated for the entire F_2 generation together, and separately by cytoplasmic origin. The phenotypic mean of each genotype was estimated by averaging replicated clones. Plasticity was calculated as the difference between warm minus cool mean phenotype. Percentage plasticity in italics represents the phenotypic change relative to warm temperature.

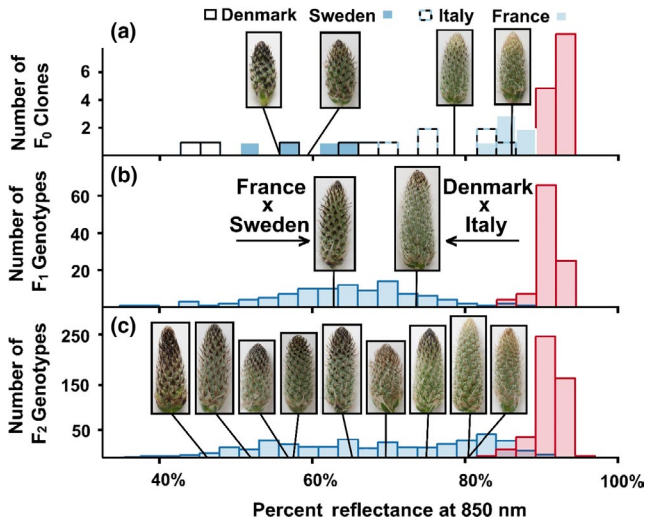

FIGURE 3 Histograms showing the percentage reflectance of preflowering spikes at 850 nm of inflorescences developed in a cool (blue) and warm (red) thermal environment. Images display visible colour variation in spikes developed at cool temperature. (a) Bars show number of clones; black lines show mean percentage reflectance of F_0 genotypes developed in cool temperature. (b) Bars show number of F_1 genotypes; black lines show mean percentage reflectance of F_1 genotypes crossed to produce F_2s developed in cool temperature. (c) Bars show number of genotypes; black lines show percentage reflectance of representative F_2 spike images developed in cool temperature

2013, we reciprocally crossed a single Danish × Italian F_1 hybrid with northern (Danish) cytoplasm and a single French × Swedish F_1 hybrid with southern (French) cytoplasm to produce the F_2 mapping population with reciprocal Danish and French cytoplasms. We used multiple clones of parental and F_1 genotypes to get enough seeds for the next generation. For both generations of crosses, the single growth chamber was set at 20°C, 16-hr day/15°C, 8-hr night. Plants were watered and fertilized with half-strength Hoagland's solution once a day. Seeds were harvested, counted and stored at room temperature until sowing (Marshall, 2017). After crossings were completed, parental and F_1 genotypes were maintained in multiple growth chambers at 20°C, 8-hr day/15°C, 16-hr night to promote vegetative growth until F_2 phenotyping began.

2.3 | Phenotyping

Because growth chamber space was limited, we grew and phenotyped two cohorts of F_2 plants subjected to the same 42-week regime. Constituting cohort 1 (2013) were 260 F_1 seeds (65 per reciprocal parental family) plus 312 F_2 seeds (156 per reciprocal F_1 family). Constituting cohort 2 (2014) were 449 F_2 seeds (226 with Danish cytoplasm and 223 with French cytoplasm from the F_1 reciprocal families). On average, members of the F_2 family grown in cohort 1 exhibited less floral reflectance plasticity (t = −2.73, df = 425, p < .007), were less reflective at warm temperature

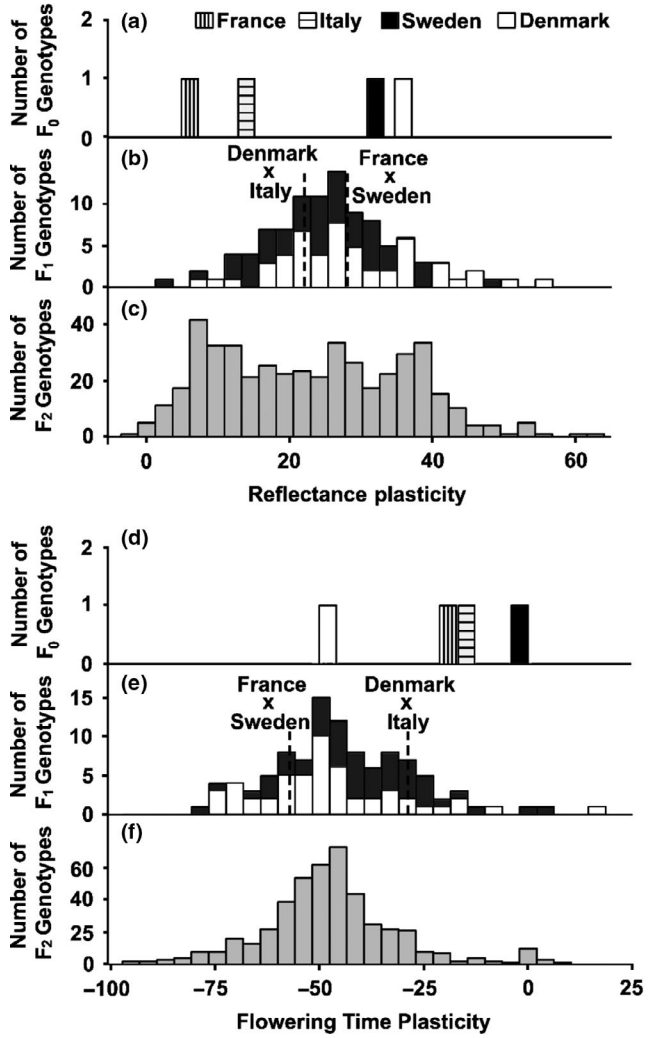

FIGURE 4 Histograms displaying temperature-sensitive plasticity of percentage reflectance of preflowering spikes at 850 nm (a–c) and flowering time (d–f) of inflorescences developed in cool and warm thermal environments (see text for details). Plasticity was calculated for each genotype as the mean trait value among clones developed in warm temperature minus the mean trait value of clones developed in cool temperature. (a) Bars show percentage reflectance plasticity of F_0 genotypes. (b) Bars show number of F_1 genotypes (grey; Southern cytoplasm, white; Northern cytoplasm); black dashed lines show reflectance plasticity of F_1 genotypes crossed to produce F_2s. (c) Bars show number of genotypes. (d) Bars show flowering time plasticity of F_0 genotypes. (e) Bars show number of F_1 genotypes (grey; Southern cytoplasm, white; Northern cytoplasm); black dashed lines show flowering time plasticity of F_1 genotypes crossed to produce F_2s. (f) Bars show number of genotypes

(t = −3.36, df = 430, p < .001), and more reflective at cool temperature (t = −2.10, df = 434, p = .036) than F_2s grown in cohort 2 (Figure S1). Additionally, the cohort 1 F_2s displayed a smaller mean flowering time plasticity (t = −3.34, df = 401, p < .001), and flowered later than cohort 2 in both warm (t = −11.48, df = 277, p < .001) and cool (t = −6.07, df = 436, p < .001) environments (Figure S1). Parental clones and clones of the F_1 hybrids used to produce the F_2 generation were interspersed among individuals in each cohort so that they could be phenotyped under the same environmental conditions as the F_2 generation.

We briefly describe the growing and treatment procedures used. Detailed information can be found in Marshall (2017). Plants were grown and cloned in growth chambers at 20°C, 8-hr day/15°C, 16-hr night to promote vegetative growth. On day 140 from the initial planting date, we moved two clones per genotype (four clones per parent), each to a different cool-temperature chamber (15°C, 8-hr day/10°C, 16-hr night), and also two each to a different warm-temperature chamber (27°C, 8-hr day/20°C, 16-hr night). In total, we used three cool and three warm chambers. On day 168, we induced flowering by extending the day length to 16-hr day/8-hr night. Then we monitored plants for emergence of flowering spikes every other day. After flowering began, we measured floral reflectance and flowering time. Floral reflectance was measured as the average of percentage light reflected at 850 nm for two spectral scans conducted on a single preflowering spike using a spectrophotometer with an integrating sphere (for methodology, see Lacey & Herr, 2005). Flowering time was the number of days from induction (day 168) to complete emergence of the first flowering spike. On day 294, data collection was completed for each cohort.

Throughout the experiment we maintained thermal differences between temperature treatments and reduced differences among other abiotic conditions. We verified temperature at plant height in each chamber daily and maintained light intensity at plant height between 300 and 325 μmol. We randomly placed clones of each genotype in different growth chambers for each temperature treatment. The cool-temperature chambers for cohort 1 were used as the warm-temperature chambers for cohort 2 and vice versa. All plants were potted in Fafard 52 mix soil, watered daily, and fertilized daily between days 42–144 and 210–294, with 0.2 tablespoons of Miracle-Gro all-purpose plant food per gallon of water. Leaves were trimmed on all plants to ~10 cm length on days 112, 196–198 and 217–220.

2.4 | Phenotypic analyses

For each genotype we calculated mean trait values in each thermal environment by averaging the trait values of clones. Trait plasticity was calculated as the warm-temperature mean trait value minus the cool-temperature mean trait value. We compared parental phenotypes in each thermal environment with one-way analysis of variance (*aov*) and Tukey's post hoc tests (*TukeyHSD*) by temperature treatment combination, with each parental genotype represented by multiple clones (R Development Core Team, 2013).

We estimated the proportion of F_2 phenotypic variance attributable to the difference between northern and southern parents for each trait at cool and warm temperature, and for trait plasticity as the proportion of variance explained (PVE) using the formula:

$$\mathrm{PVE} = \frac{\sigma^2_{F_2} - \sqrt{\sigma^2_N \times \sigma^2_S}}{\sigma^2_{F_2}}$$

where σ_N^2 and σ_S^2 represent variances of parents from northern and southern populations, respectively, and $\sigma_{F_2}^2$ is the F_2 variance (Mahmud & Kramer, 1951). We estimated parental variances (σ_N^2 and σ_S^2) for cool- and warm-temperature conditions separately from the mean trait value of clones of northern and southern parental genotypes. We calculated parental variances for plasticity by estimating multiple plasticity values for each genotype. This was done by subtracting the mean trait value of a randomly selected clone in cool conditions from the mean trait value of a randomly selected clone in warm conditions without resampling. Therefore, for our variance calculations of plasticity, each parental genotype contributed a number of plasticity estimates equal to the fewest number of clones measured in either environment (Table 1; Table S1).

We tested F_2 trait distributions for normality with the Shapiro–Wilk test shapiro.test in R/stats and kurtosis by Pearson's kurtosis statistic kurtosis in R/moments (Komsta & Novomestky, 2015; R Development Core Team, 2013). We calculated the genotypic variance and trait correlations using genotypic mean trait values and trait plasticities with the cor and cov functions in R (R Development Core Team, 2013).

2.5 | Genotyping

We collected 100 mg of young leaf tissue from 465 individual genotypes (four F_0, two F_1, 459 F_2) and stored it at −80°C until extractions were performed. DNA was extracted using the MasterPure plant leaf DNA purification kit. The integrity of high-molecular-weight DNA bands was verified visually on 1% agarose gels run in 1× TAE buffer, stained with 0.2 μg/ml ethidium bromide and viewed with the Bio-Rad ChemiDoc XRS system.

Plantago lanceolata has neither a sequenced genome nor readily available genetic markers. Therefore, we used the ddRADseq protocol (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012) to develop reproducible genetic markers evenly spread across the genome. Markers were used to produce a genetic recombination map. Initially, we selected four nonmethylation-sensitive enzymes with an optimal reaction temperature of 37°C to determine which restriction enzymes would be appropriate for this project. Two were "common cutters" with 4-nucleotide recognition sites, *Mse*I and *Msp*I, and two were "rare cutters" with 6-nucleotide recognition sites, *Eco*RI and *Pst*I. We performed single digestions (each restriction enzyme alone) and double digestions (each combination of common + rare cutter) on genomic DNA from each of the F_0 parents (for details see Appendix S1). We subjected digested DNA samples to a dilution series and ran them on an Agilent 2100 Bioanalyzer High Sensitivity chip. The number of sequenceable fragments produced from each combination of restriction enzymes was estimated using the methods described in Peterson et al. (2012). After digestion with *Eco*RI and *Msp*I, we estimated a size selection window of 200–400 bp to produce ~38,000 sequenceable fragments per individual. Therefore, to capture DNA fragments of 200–400 bp ligated to 120 bp of adapters, we used a size selection window of 320–520 bp for library preparation.

We sent genomic DNA samples of 465 individual genotypes (four F_0, two F_1, 459 F_2) to the genomics core lab at Texas A&M University Corpus Christi for library preparation where solid phase reversible immobilization (SPRI) size selection was used to purify high-molecular-weight genomic DNA. To ensure a sufficient number of markers was obtained from each of the parents, DNA was included from two separate extractions of F_0 parents. Illumina library preparation was conducted using the restriction enzymes *Eco*RI and *Msp*I with a size selection window of 320–520 bp. For each run, 100-bp paired-end sequencing was performed on a single Illumina lane of 196 pooled individuals. We estimated this volume to produce ~38,000 reads per individual with 40× coverage.

The number of total and unique reads derived from the Swedish parent and French–Swedish F_1 hybrid were much lower than was obtained from the other parents. Therefore, we conducted a third library preparation and second sequencing run that included these genotypes, and some F_2s that contained a low number of reads from the initial run. The second sequencing run increased the number of markers obtained from some individuals, while others, including the Swedish F_0 parent and French–Swedish F_1 hybrid remained low (i.e., <2,000 unique reads, Table S2).

2.6 | Linkage mapping

We used the following workflow in STACKS version 1.35–1.37 to process ddRADseq reads and produce the genetic markers (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011; Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). We filtered raw reads from each sequencing run to remove erroneous and low-quality reads, and then demultiplexed the reads (see Appendix S1 for details). Then we sorted and scanned reads from each individual with a minimum of 5× coverage and maximum of two alleles per locus against a catalogue of loci from F_0 and F_1 parental genotypes and exported matching reads for each genetic locus (marker). We eliminated markers that (a) could not be traced to F_0 parents, (b) did not display allelic differences between F_1 parents, (c) were scored in less than 70% of the 104 F_2s with highest coverage and (d) had segregation ratio p-values ≤.0001. Remaining markers were used for recombination mapping.

We conducted recombination mapping using the 104 F_2s (22.6%) with the highest sequence coverage. Based upon the alleles identified and their segregation patterns, each genetic marker was categorized as either fully informative (segregating 1:1:1:1; Type A) or partially informative (segregating 1:2:1; Type B, or 1:1; Type D), as described by Wu, Ma, Painter, and Zeng (2002). Then, we removed markers with extremely skewed segregation ratios (i.e., $p < .0001$ from chi-squared tests of observed vs. expected segregation ratios of each marker). Filtering produced a set of 555 genotyped markers that were used to create a genetic recombination map.

We used the Kosambi mapping function in the R/OneMap 2.0–4 package to calculate marker order and genetic distance (Kosambi, 1943; Margarido, Souza, & Garcia, 2007; R Development Core Team, 2013). De novo linkage mapping proceeded in three phases based upon marker segregation ratio *p*-values. First, we grouped markers with segregation *p*-values ≥.05 using recombination frequencies ≤ 0.40 and logarithm of the odds (LOD) scores ≥ 4.0. Within a linkage group (LG), we estimated preliminary marker order using the order.seq function. We evaluated the resulting order using the recombination fraction matrix (Figure S2). Markers that did not show recombination frequencies monotonically increasing with distance from the diagonal of the recombination frequency heat map were relocated using the try.seq and make.seq functions, or they were removed. Once all markers within the LG displayed a monotonic recombination frequency pattern, we forced each other marker initially grouped with those markers onto the LG, one at a time, to determine if they fit soundly at any position along the lineage group. If forcing a marker onto the LG resulted in map expansion or violation of monotony, we relocated or removed it. Second, we added markers with segregation ratio *p*-values ≥.01, and third, we added markers with *p*-values ≥.0001 to LGs using the same criteria (map expansion and violation of monotony). Finally, we forced all remaining markers that did not fit soundly on any of the LGs together onto a single, separate LG and evaluated position using the same criteria. Once all 555 markers were tested, we evaluated the order of each LG using the ripple.seq function with a sliding window size of 4, LOD threshold of 2.0 and tolerance value of 0.1. We examined alternative orders that produced lower LOD scores for map expansion and violation of monotony along the LG. In cases when reordering did not produce a better overall linkage map, we removed the least informative markers.

2.7 | Marker distribution and genome coverage

We calculated average marker spacing *s* by dividing the summed length of all LGs by the number of marker intervals in the final linkage map. We estimated the length of each LG *i* as $G_i = M_i + 2s$, where M_i is the map distance between terminal markers of LG *i*. The expected distance between the chromosome end and the terminal marker is *s* under a uniform probability distribution. We calculated the estimated genome length *L* by summing the lengths of all six LGs. The number of markers m_i in LG *i* would be a sample from a Poisson distribution with parameter $\lambda_i = mG_i/\Sigma_i G_i$, where *m* is the total number of markers, if the marker density underlying all chromosomes were the same (Remington, Whetten, Liu, & O'malley, 1999). We evaluated the probabilities $P(X \le m_i)$ and $P(X \ge m_i)$ under the cumulative Poisson distribution as $\lambda_i = m_i G_i/L$ (Remington et al., 1999). We compared marker density with expected marker density under the Poisson distribution to evaluate marker distribution among LGs on the final linkage map. We estimated the proportion of the genome *c*, within 10 cM, and within 20 cM of a marker, using the formula:

$$c = 1 - e^{2dn/L}$$

where *d* is the specified distance (i.e., 10 or 20 cM), *n* is the number of markers and *L* is the estimated genome length, assuming a random marker distribution (Lange & Boehnke, 1982).

2.8 | QTL mapping

For each trait, floral reflectance and flowering time, we carried out the following analyses separately in each thermal environment (i.e., cool and warm), and for mean thermal plasticity. We performed genome-wide interval mapping scans with the scanone function in the R/qtl package to identify genomic regions underlying phenotypic variation in R 3.2.3 (Broman, Wu, Sen, & Churchill, 2003; R Development Core Team, 2013). We analysed reciprocal progeny together and included cytoplasm origin as an additive covariate. We used 1,000 permutations to determine genome-wide LOD thresholds of $p = .05$ for each trait (Churchill & Doerge, 1994).

To estimate the genetic architecture of each trait we used the *makeqtl* and *fitqtl* functions. We made all putative QTL peaks with LOD ≥ 3.0 identified by *scanone* into a QTL with *makeqtl*. We used two methods to test the significance of each putative QTL, cytoplasm type (as an additive covariate), and two-way interactions between QTLs and between QTL and cytoplasm type. First, we placed all putative QTLs and the cytoplasm covariate into an additive model containing all main QTL effects and all two-way interactions. The general form of the model was:

$$\text{trait} = Q_i + Q_{ii} + \text{Cytoplasm} + Q_i * Q_{ii} + Q_i * \text{Cytoplasm} + Q_{ii} * \text{Cytoplasm}$$

where $Q_i = \text{QTL}_1$ and $Q_{ii} = \text{QTL}_2$. Then we executed *fitqtl* on the model. We performed an iterative stepwise reduction by removing terms, one at a time, starting with the highest *p*-value>.05. This process was repeated until all terms in the model reached *p*-values ≤.05. Second, we evaluated *fitqtl* models for each trait by iterative stepwise addition. Here we began with only the putative main effect QTLs and cytoplasm terms in the model. We first reduced the model until all terms reached *p*-values ≤.05. Then we added two-way interactions, one at a time, and retained significant terms. To avoid overlooking important interactions when an interaction was identified and added to the model, we also tested each two-way interaction in the model with previously added interactions excluded. Both stepwise reduction and stepwise addition methods for evaluating the genetic architecture with *fitqtl* models produced the same "best" genetic architecture model for each trait-by-environment combination.

We partitioned each QTL that contributed to the genetic architecture into QTL effects of northern versus southern alleles as additive effects, dominance effects, and the deviation of the two northern/southern heterozygous classes from the mean of the northern/southern heterozygotes using a custom glm script in R that partitions the effects of one QTL at a time from outcross F_2 data (Remington, Leinonen, Leppälä, & Savolainen, 2013). Partitioning allowed us to estimate the magnitude and direction of QTL effects contributing to the genetic architecture of each trait. We used this information to determine if similar effects were found between the QTLs underlying the trait plasticity and trait variation in either thermal environment. Additionally, using

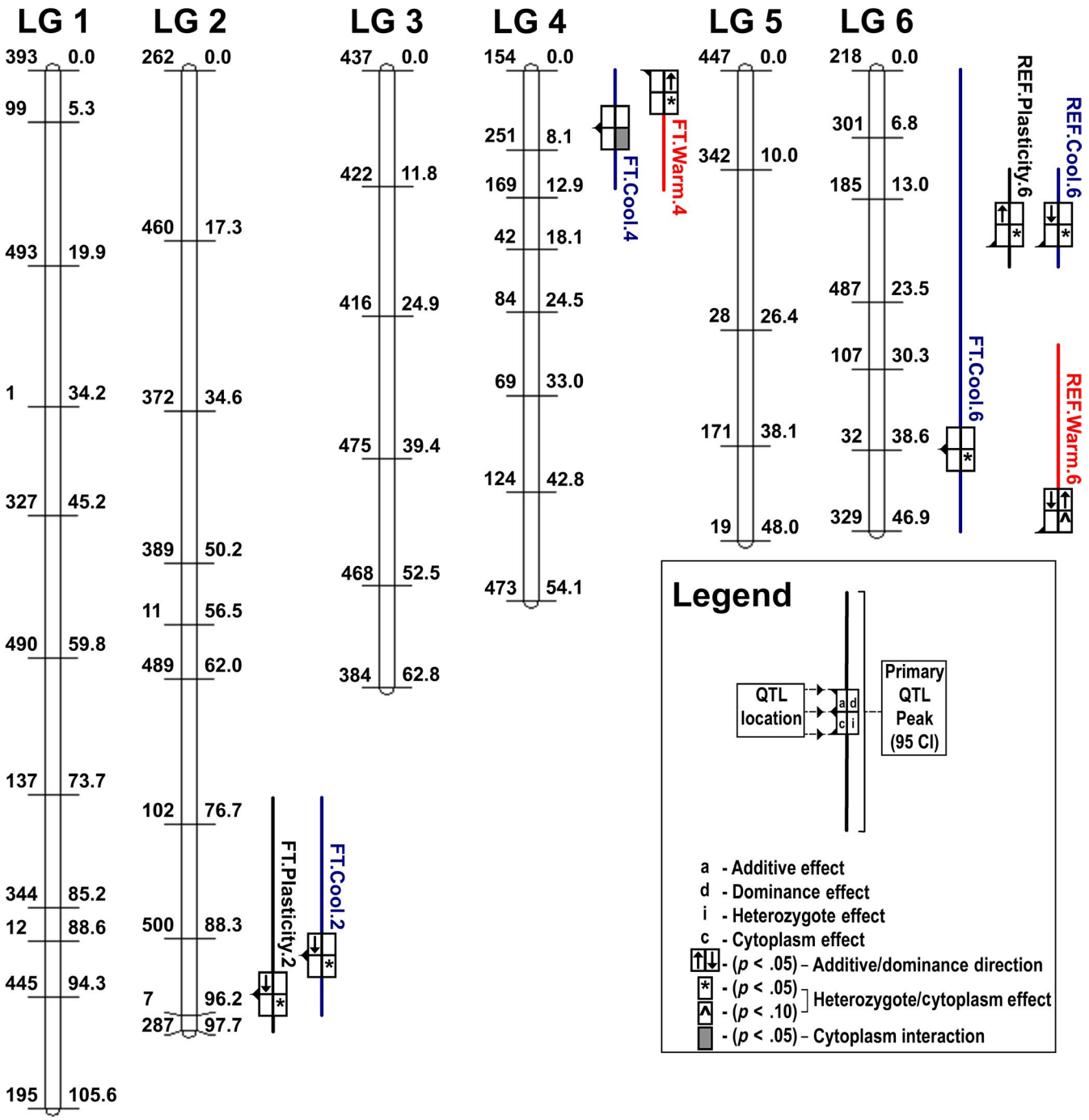

FIGURE 5 QTLs displaying a significant effect on trait values in cool and warm environments, and trait plasticity for floral reflectance and flowering time. QTL peak locations and Bayesian 95% credible intervals (solid lines) are shown to the right, and genetic markers are shown to the left of each linkage group (LG). Significant QTLs and interactions were identified using the fitqtl function in R/QTL. Each QTL was partitioned into additive (a), dominance (d), difference between heterozygous classes (i), cytoplasmic (c) and cytoplasmic interactions (shaded boxes) in separate generalized linear models. Arrows indicate the significance and direction of additive and dominance effects of alleles from northern (Danish and Swedish) parents. Asterisk (*) and hat (^) symbols indicate the significance of difference between heterozygous classes and cytoplasmic effects. QTLs are labelled as **[trait].[environment].[LG]**; **trait**: floral reflectance (REF) or flowering time (FT); **environment**: cool, warm or plasticity; and **LG**: numbered 1–6

this script, we examined whether cytoplasm contributed a significant additive effect at each QTL locus, and whether the following interactions were significant: cytoplasm by additive, cytoplasm by dominance and cytoplasm by difference between northern/southern heterozygous classes. We estimated and plotted genotypic means and standard errors of significant QTL-by-cytoplasm interactions with the effectplot function in R/QTL (Broman et al., 2003; R Development Core Team, 2013).

Each QTL that contributed to the genetic architecture was labelled as **[trait].[environment].[LG]** and abbreviated as follows, **trait**: [floral reflectance (REF), flowering time (FT)]; **environment**: [cool,

TABLE 2 Analysis of variance and Tukey's post hoc test results comparing floral reflectance and flowering time of parental genotypes from Denmark (D), Sweden (S), France (F) and Italy (I) grown in cool and warm environments

Trait	Temperature	*df*	MS	*F*	*p*
Floral reflectance	Cool residuals	3	1,129.6	27.28	**1.00E−06**
		17	41.4		
		Contrasts	**Difference**	**95% CI (lower/upper)**	***p***
		D–I	−22.8	(−33.88/−11.72)	**1.04E−04**
		F–I	7.53	(−3.03/18.09)	.217
		S–I	−19.04	(−30.85/−7.23)	**.001**
		F–D	30.33	(19.25/41.41)	**2.90E−06**
		S–D	3.76	(−8.51/16.03)	.819
		S–F	−26.57	(−38.38/−14.76)	**3.61E−05**
		df	**MS**	***F***	***p***
	Warm residuals	3	1.24	0.91	.472
		10	1.37		
		df	**MS**	***F***	***p***
Flowering time	Cool residuals	3	4,084	10.08	**3.43E−04**
		19	405		
		Contrasts	**Difference**	**95% CI (lower/upper)**	***p***
		D–I	39.25	(6.99/71.51)	**.014**
		F–I	4.17	(−30.1/38.43)	.986
		S–I	60	(22.04/97.96)	**.001**
		F–D	−35.08	(−65.65/−4.52)	**.021**
		S–D	20.75	(−13.91/55.41)	.359
		S–F	55.83	(19.3/92.36)	**.002**
		df	**MS**	***F***	***p***
	Warm residuals	3	2,961.5	113.7	**1.79E−07**
		9	26.1		
		Contrasts	**Difference**	**95% CI (lower/upper)**	***p***
		D–I	6.25	(−7.55/20.05)	.522
		F–I	0.85	(−9.84/11.54)	.994
		S–I	73.75	(59.95/87.55)	**2.00E−07**
		F–D	−5.4	(−18.73/7.93)	.605
		S–D	67.5	(51.57/83.43)	**1.60E−06**
		S–F	72.9	(59.57/86.23)	**2.00E−07**

Note: In each thermal environment genotypes were represented by multiple clones. Column headers indicate degrees of freedom (*df*), mean square (MS), *F* statistic (*F*), *p*-value (*p*), post hoc contrast (Contrasts), mean difference (Difference) and 95% confidence interval (CI).

warm or plasticity]; and **LG**: [linkage group numbered 1–6 from longest to shortest, corresponding to the genetic map] (Figure 5).

3 | RESULTS

3.1 | Phenotypic patterns

We observed latitudinal differences in the degree of thermal plasticity for both reproductive traits (Marshall, Remington, & Lacey, 2019b). All parents produced highly reflective flowers in the warm "southern" environment, but northern parents significantly and substantially reduced reflectance more than did southern parents in the cool "northern" environment (Tables 1 and 2; Figure 3). Swedish and Danish parents responded similarly to temperature, as did French and Italian parents.

The pattern for flowering time was more complicated. At cool temperature, northern parents flowered significantly later than did southern parents (Tables 1 and 2). However, in warm temperature the Swedish parent flowered significantly later than the

TABLE 3 Pearson correlation coefficients and *p*-values (lower left), covariances (upper right) and variances (diagonal) of floral reflectance and flowering time in the F_2 mapping population

	Floral reflectance			Flowering time		
	Cool	Warm	Plasticity	Cool	Warm	Plasticity
Floral reflectance						
Cool	**177**	7.6	−172.6	19.37	.85	−17.71
Warm	.29; <.001	**3.9**	−3.71	9.39	.94	−6.97
Plasticity	−.99; <.001	−.15; .002	**168.9**	−9.94	.11	10.25
Flowering time						
Cool	.10; .025	.05; .272	−.05; .312	**213**	78.33	−136.1
Warm	.03; .53	.04; .438	.00; .938	.39; <.001	**183.5**	107.64
Plasticity	−.10; .043	−.04; .394	.05; .283	−.61; <.001	.52; <.001	**243.7**

Note: Genotypic means were calculated as the average value of clones developed in cool and warm environments. Plasticity was calculated for each genotype as the difference between the warm minus cool mean phenotype.

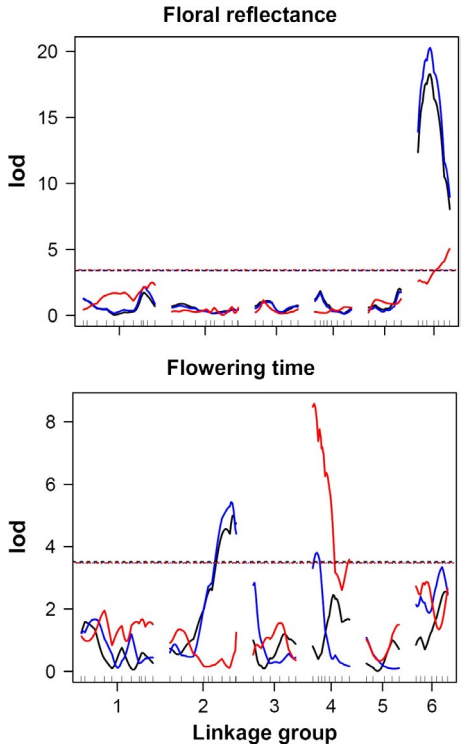

FIGURE 6 LOD profiles for floral reflectance and flowering time are shown for trait values in cool (blue) and warm (red) environments, and trait plasticity (black). Horizontal dashed lines represent genome-wide p = .05 significance thresholds

others, which all flowered more quickly than they did at cool temperature (Table 2). Thus, there was a strong genotype-specific difference; the Swedish parent was thermally insensitive (Table 1). Consequently, flowering time plasticity was lowest in the Swedish parent, highest in the Danish parent and intermediate in the southern parents (Table 1).

For both traits, thermal plasticity differences between northern and southern parents showed evidence of a genetic basis (Table S1). Mean F_2 plasticity resembled the midparent values, and the F_2 phenotypic distributions were wider than the phenotypic ranges of the parent genotypes (Figure 4). Floral reflectance at both temperatures and flowering time in warm temperature displayed the same pattern. However, the F_2s were less variable in cool-temperature flowering time than were the parents, resulting in a negative estimate of PVE (Table S1). Most F_2s (>99%) showed reduced reflectance and delayed flowering in cool relative to warm temperature, similar to parental genotypes (Table 1; Figure 4).

3.2 | F_2 genotypic correlations

For each trait, we detected statistically significant genotypic correlations between thermal plasticity and the trait's value in both cool and warm temperatures (Table 3). However, correlation values were strong for only a few cases. Plasticity in floral reflectance was very strongly negatively correlated with reflectance in cool temperature (r = −0.99) and only weakly correlated with reflectance in warm temperature (r = 0.29). Thus, thermal plasticity in floral reflectance was primarily driven by decreased reflectance in the cool environment. The correlations between plasticity in flowering time and flowering in cool and warm temperatures were fairly equal (−0.61 and 0.52, respectively) suggesting that responses to both temperatures contribute to thermal plasticity in flowering time.

3.3 | Linkage mapping

Illumina sequencing of the ddRADseq libraries produced 69 k to 2.7 M reads with mean coverage of 13–31× in F_0 parents, 24 k to 1 M

TABLE 4 Overall QTL models from *fit.qtl* analysis in R/QTL for each trait examined in cool and warm temperature, and trait plasticity

A. REFLECTANCE PLASTICITY				
Best model = y ~ REF.Plasticity.6				
Model parameters	**LOD**	**%var**	***p* (Chi)**	***p* (*F*)**
REF.Plasticity.6	18.286	17.563	<.0001	<.0001***
B. REFLECTANCE COOL				
Best model = y ~ REF.Cool.6				
Model parameters	**LOD**	**%var**	***p* (Chi)**	***p* (*F*)**
REF.Cool.6	20.585	19.012	<.001	<.001***
C. REFLECTANCE WARM				
Best model = y ~ REF.Warm.6				
Model parameters	**LOD**	**%var**	***p* (Chi)**	***p* (F)**
Ref.Warm.6	5.053	5.084	3.55E−05	3.87E−05***
D. FLOWERING TIME PLASTICITY				
Best model = y ~ FT.Plasticity.2				
Model parameters	**LOD**	**%var**	***p* (Chi)**	***p* (F)**
FT.Plasticity.2	4.999	5.108	4.00E−05	4.37E−05***
E. FLOWERING TIME COOL				
Best model = y ~ FT.Cool.2 + FT.Cool.4 + FT.Cool.6 + Cytoplasm + FT.Cool.4 × Cytoplasm				
Model parameters	**LOD**	**%var**	***p* (Chi)**	***p* (*F*)**
Full Model	14.033	13.488	7.72E−09	1.30E−08***
FT.Cool.2	4.515	4.128	<.001	1.57E−04***
FT.Cool.4	5.711	5.255	<.001	2.65E−04***
FT.Cool.6	2.819	2.555	.005	.006**
Cytoplasm	2.278	2.059	.033	.037*
FT.Cool.4 × Cytoplasm Interaction	2.259	2.042	.015	.018*
F. FLOWERING TIME WARM				
Best model = y ~ FT.Warm.4				
Model parameters	**LOD**	**%var**	***p* (Chi)**	***p* (*F*)**
FT.Warm.4	8.581	8.425	1.35E−08	1.57E−08***

Note: Best models were determined when all model parameters achieved $p < .05$. QTLs are labelled as **[trait].[environment].[LG]**, **traits**: floral reflectance (REF), flowering time (FT); **environments**: cool, warm or plasticity; and **LG**: numbered 1–6.

***$p < .001$.

**$p < .01$.

*$p < .05$.

reads with mean coverage of 13–29× in F_1 parents, and a mean of 597 k reads at 16× coverage in F_2s (Table S2).

The bioinformatics processing steps produced 11,295 markers from forward reads and 10,387 markers from reverse reads (Marshall, Remington, & Lacey, 2019a). Eliminating noninformative markers reduced the number of markers to be used for mapping to 555. Of the 555 markers used for genetic mapping, 232 displayed segregation ratio *p*-values ≥.05, 122 displayed ratio *p*-values between .05 and ≥.01, and 201 displayed ratio *p*-values between .01 and ≥.0001. The 555 markers represented three segregation patterns. Three markers were fully informative and segregated in a 1:1:1:1 ratio (Type A). The remaining markers were partially informative; 426 segregated in a 1:2:1 ratio (Type B) and 126 segregated in a 1:1 ratio (Type D).

The final genetic linkage map contained 47 markers along 6 LGs with a combined length of 415.1 cM Kosambi (Figure 5; Table S3). The average spacing between markers was 10.1 cM, a density sufficient to provide nearly full resolution of QTL intervals given the limitations of linkage-based mapping for inferring QTL locations (Darvasi, Weinreb, Minke, Weller, & Soller, 1993). Of the genetic markers in the final linkage map, one was Type A, 36 were Type B and 10 were Type D. Markers with skewed segregation ratios tended to cluster together, and one of the six LGs (LG3) consisted entirely of highly skewed markers (Figure 5; Table S3). Assuming that markers were evenly spaced and each LG corresponded to a single chromosome, the average distance between chromosomal ends and terminal markers equalled the average marker spacing of

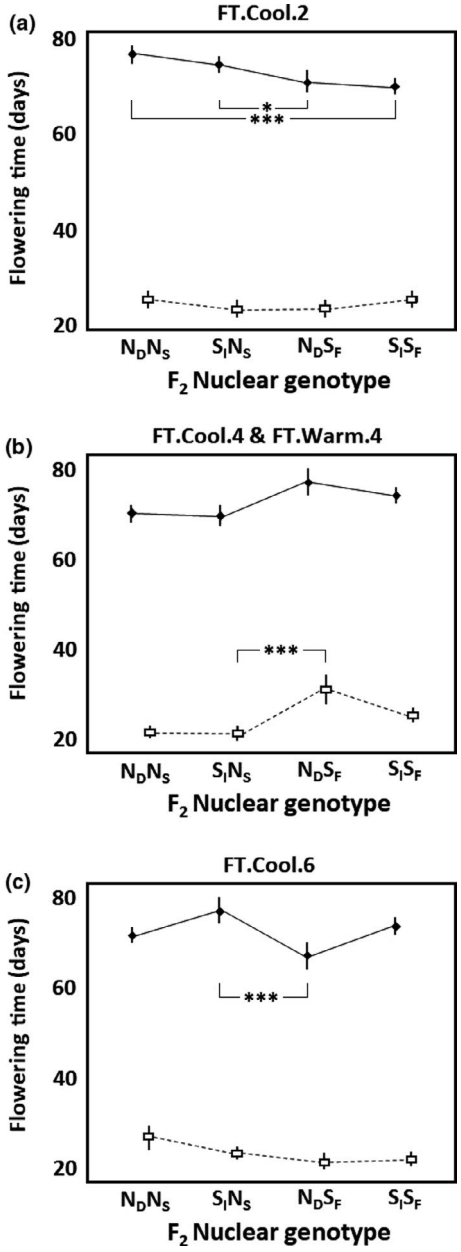

FIGURE 7 Genotypic means (±*SE*) of F_2s at flowering time QTLs: (a) FT.Cool.2, (b) FT.Cool.4 and FT.Warm.4, and (c) FT.Cool.6. Symbols indicate trait values measured in cool (filled diamond) and warm (open rectangle) thermal environments. QTLs are labelled as **[trait].[environment].[LG]**, **trait**: flowering time (FT); **environment**: cool or warm; and **LG**: numbered 1–6. Allele designations indicate inheritance from F_0 parent; N_D = Danish, N_S = Swedish, S_I = Italian, S_F = French

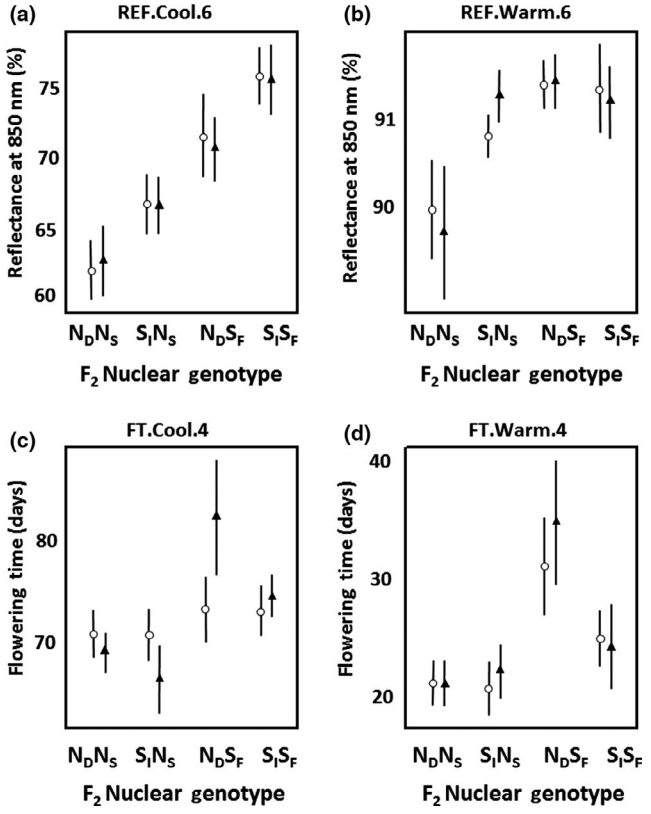

FIGURE 8 Effect of cytoplasms on genotypic means (±*SE*) of reciprocal F_2s at the QTLs REF.Cool.6 (a), REF.Warm.6 (b), FT.Cool.4 (c) and FT.Warm.4 (d). Symbols represent Danish (open circles) and French (filled triangles) cytoplasmic genomes. QTLs are labelled as **[trait].[environment].[LG]**, **trait**: floral reflectance (REF), flowering time (FT); **environment**: cool or warm; and **LG**: numbered 1–6. Allele designations indicate inheritance from F_0 parent; N_D = Danish, N_S = Swedish, S_I = Italian, S_F = French

10.1 cM. Based on these assumptions, the estimated map length was 536.3 cM.

We did not detect significant differences in marker density among LGs because Poisson probabilities for deviations of m_i from λ_i in either direction were greater than 0.329 (Table S4). We estimated that 82.7% of the genome was within 10 cM of a genetic marker; 97.0% was within 20 cM (Lange & Boehnke, 1982).

3.4 | QTL mapping

We detected two QTL regions on different parts of LG6 affecting floral reflectance (Figures 5 and 6; Table 4). One QTL with a peak at 18 cM on LG6 explained most of the difference in reflectance between northern versus southern parents at cool temperatures, with northern alleles reducing reflectance leading to darker flowers. The region had no effect at warm temperatures. As a consequence, additive effects of plants with two northern versus two southern alleles at this QTL region also explained 78% of the increased thermal plasticity in floral reflectance in northern parents versus southern parents (Table S5). In addition to additive effects, this QTL region displayed a significant difference between northern/southern heterozygote classes in reflectance at cool temperature. Heterozygotes with the Swedish/Italian genotype produced darker, less reflective flowers in cool temperature, and exhibited greater thermal plasticity for floral reflectance than did Danish/French heterozygotes (Table S5). A second QTL region, near the opposite end of LG6, had much smaller effects on floral reflectance, and these effects were significant only at warm temperatures (Table S5).

In contrast, we detected three significant or probable QTL regions with relatively small effects on flowering time in cool and/or warm temperatures, each on a separate LG (LG2, 4 and 6; Figures 5 and 6; Table 4). At the LG2 QTL region, F_2 plants with two northern alleles flowered later, on average, in cool temperatures than did plants with two southern alleles, and Swedish/Italian heterozygotes flowered later than did Danish/French heterozygotes (Figure 7; Table S5). The LG2 region had no detectable effect under warm temperature. This led to a QTL peak in the same region with corresponding effects on flowering time plasticity (Table S5; Figure 6). A probable QTL region on LG6, which did not quite reach the genome-wide $p = .05$ LOD threshold, was detected only under cool temperatures, with effects limited to differences between the two northern/southern heterozygote classes. Swedish/Italian heterozygotes at this location flowered later than did Danish/French heterozygotes under cool temperatures, but warm-temperature and plasticity effects were not detected.

On LG4, flowering time QTLs were detected at similar locations under both warm and cool conditions. Under warm temperatures Danish/French heterozygotes flowered significantly later than did Swedish/Italian heterozygotes (Figure 7; Table S5). Similar patterns were seen under cool temperatures, but the individual genotypic contrasts were not significant. Under warm temperature, cytoplasm did not affect flowering time in the Danish/French heterozygotes (Figure 8d). However, there was a significant interaction between cytoplasm type and the LG4 QTL genotypes under cool temperatures (Figure 8c; Table S5). Under cool temperature, plants with French cytoplasm showed delayed flowering in Danish/French heterozygotes, similar to the pattern seen with warm temperatures, but the Danish cytoplasm accelerated flowering of heterozygotes with the Danish/French QTL genotype. This resulted in a significant reduction in the flowering time difference between Danish/French and Swedish/Italian heterozygotes from 16 days in the French cytoplasmic background to <3 days in the Danish cytoplasm (Figure 8c; Table S5).

All flowering time QTL effects were relatively small compared to the flowering time differences between the parents. The largest additive effect was that of the LG2 QTL region under cool temperatures, for which the flowering time difference between plants with two northern versus two southern alleles was about 16 days, constituting 33% of the mean difference between northern versus southern parents (Table S5).

4 | DISCUSSION

This study identified substantial genetic differences in genetic architecture of floral reflectance, flowering time and thermal plasticities between individuals from northern and southern European populations. The results for thermal plasticity in floral reflectance were consistent with a single gene explaining most of the differences between populations. One large-effect QTL on LG6 was associated with both thermal plasticity in reflectance and reflectance at cool temperature, and the overlap of QTLs was complete. This large-effect QTL largely explains the strong phenotypic correlation ($r = -0.99$) that we observed between thermal plasticity of floral reflectance and reflectance at cool temperature in the F_2 plants. The additive effect of substituting both northern alleles for southern alleles at this QTL explained 78% of the difference in mean plasticity values between our northern and southern parents. Furthermore, the significant differences between the two F_2 northern/southern heterozygotes at the plasticity QTL suggest strongly that the latitudinal and within-latitudinal variation in thermal plasticity reflect an allelic series in thermal sensitivity to cool temperatures. In *Petunia* flowers, an allelic series at the *An1* regulatory gene that promotes anthocyanin biosynthesis produces a similar thermal response (i.e. anthocyanin accumulation in cool- vs. warm-developed flowers), and explains the continuous variation of flower colour (Gerats, Farcy, Wallroth, Groot, & Schram, 1984). Whether or not the LG6 reflectance plasticity QTL region is actually due to the effects of a single gene versus multiple neighbouring genes remains to be established. The LG6 region does not explain the entire difference in reflectance plasticity between the northern and southern parents. This suggests that a polygenic component to reflectance differences still exists, which could include epistatic interactions with the LG6 region.

Rockman (2012) noted that pigmentation (e.g., via anthocyanin biosynthesis in plants) in natural populations has an atypically simple genetic architecture compared to other traits, possibly because it is generally regulated by simple molecular pathways. However, recent research shows that regulation of the anthocyanin biosynthetic pathway is not simple (see review by Gu et al., 2019). Moreover, the large-effect LG6 QTL region seems likely to regulate a complex suite of traits integrating temperature responses, anthocyanin production and other undetermined reflectance mechanisms. Reflectance plasticity occurs in both the visible and NIR regions, and thermal responses in these regions are highly correlated in *Plantago lanceolata* and other *Plantago* species (Anderson, Lovin, et al., 2013; Lacey & Herr, 2005). Cool-temperature regulation of anthocyanin accumulation explains the thermal plasticity in the visible region (Stiles et al., 2007). However, the molecular pathway underlying change in the NIR is unknown, as anthocyanins do not absorb NIR radiation (Gitelson, Merzlyak, & Chivkunova, 2001; Merzlyak, Chivkunova, Solovchenko, & Naqvi, 2008). Also, because colour is seldom measured beyond the visible region, information about whether temperature modifies NIR reflectance in most other plant species is lacking. Our reflectance measurements were made at 850 nm, thus demonstrating QTL effects in the NIR spectrum. Divergent selection on complex pigmentation patterns has been found to involve simple genetic architectures consisting of large-effect genes in other instances, including deer mice coat colour patterns as adaptations to different soil colours (Linnen et al., 2013) and *Heliconius* butterfly wing patterns under Müllerian mimickry (Supple et al., 2013). Thus, the atypically simple genetic architecture of pigmentation noted by Rockman (2012) may extend to complex pigmentation patterns.

Flowering time showed a different and more complex pattern that lacked large-effect QTLs under either warm or cool conditions.

Although we detected one QTL for thermal plasticity that fell within the primary QTL peak for cool-temperature flowering, the additive effect of substituting both northern alleles for southern alleles in the LG2 QTL region explained only a small fraction of the later cool-temperature flowering in the northern parents. Additional QTLs for both warm- and cool-temperature flowering time were found, but none explained the majority of divergence among parents. Also, the F_2 plants with Swedish alleles did not flower later, as would be expected from the constitutive late flowering in the Swedish parent. Thus, the data suggest that undetected loci influenced a substantial amount of the differences in flowering time plasticity between parent genotypes. Also, QTL effects mainly involved heterogeneity among northern or southern alleles, and in one case (on LG4) a strong cytonuclear interaction under cool conditions. This further suggests that additional undetected QTLs and/or epistatic interactions must explain the late flowering of the Swedish parent under warm conditions. Parental effects are unlikely to explain late flowering because all parents were themselves the offspring of parents that had been raised and that had reproduced in similar environmental conditions (Lacey et al., 2010). Multiple genes in a complex network help regulate flowering time in response to photoperiod and temperature in *Arabidopsis thaliana* (e.g., Johansson & Staiger, 2014; Méndez-Vigo et al., 2016; Song et al., 2017; Song, Ito, & Imaizumi, 2013), and a similarly complex network appears to underlie latitudinal variation in flowering time in this species.

In addition to possible differences in the inherent regulatory complexity of the two traits, differences in selective regimes might explain the contrast in genetic architecture between reflectance and flowering time. Divergent selection has either been established or is suspected in cases where large-effect genes have been implicated in trait variation (Colosimo et al., 2005; Frankel et al., 2012; Lamichhaney et al., 2016; McGregor et al., 2007; Shapiro et al., 2004), and reflectance plasticity in *P. lanceolata* is consistent with this pattern of selection (Lacey et al., 2012, 2010; Marshall et al., 2019). Flowering time presents a more nuanced situation. In a cross between Swedish and Italian *A. thaliana* ecotypes, northern QTL alleles also led to later flowering, although in some cases they were associated with lower fitness in Sweden-like growing conditions (Dittmar et al., 2014). Flowering time in *P. lanceolata*, likewise, might not be under strong divergent selection along a latitudinal gradient, although for a different reason. Unlike *A. thaliana*, *P. lanceolata* is thermally plastic for floral reflectance (Lacey & Herr, 2005; Lacey et al., 2010). Thus, *P. lanceolata* has two potential mechanisms by which to influence the thermal microenvironment of its reproductive tissues: regulating flowering time and regulating reflectance. It is possible that the strong selection for differences in reflectance plasticity at the northern portion of its range may have led to a weaker selection gradient for flowering time because reflectance plasticity can extend/maintain a seasonal window for successful reproduction. When genotypes ranging in floral reflectance plasticity were transplanted into the field at different times during the flowering season, high-plasticity individuals set more seeds than did low-plasticity individuals during the cool portion of the season, with no measurable cost in the warm portion (Lacey et al., 2012).

Our QTL data showed no evidence of pleiotropy in QTLs affecting reflectance and flowering time in *P. lanceolata*. Rather, the data strongly suggested that the thermoregulation of floral reflectance and of flowering time involve different molecular pathways. The plasticity QTLs did not colocalize, and the genetic correlations between traits and between the thermal plasticities of these traits were weak. One result is that different patterns of thermal plasticity in this pair of traits have apparently been free to evolve independently without constraining each other. Both of our southern populations have evolved a moderate degree of thermal plasticity in flowering time but have constitutively high floral reflectance. Conversely, the Swedish population has high thermal plasticity for floral reflectance but constitutively late flowering, while the Danish population has high thermal plasticity for both traits. Two molecular pathways that independently could thermoregulate anthocyanin accumulation have recently been identified in *A. thaliana* and in apple. Phytochrome B (PHYB), an important regulator of light- and temperature-mediated flowering time (Gu et al., 2019; Legris, Nieto, Sellaro, Prat, & Casal, 2017; Song et al., 2017) helps to regulate flowering via its effect on the COP1 (constitutive photomorphogenic 1) ligase. However, an indirect effect of this is that PHYB may also contribute to anthocyanin accumulation in leaves (Wu et al., 2018). COP1 has been shown in *A. thaliana* to repress MYB1/10 transcription factors, which are positive regulators of the anthocyanin biosynthetic pathway (ABP) (Li, Ban, et al., 2012a; Qiu, Li, Jean, Moore, & Chen, 2019; Zhou et al., 2019). Secondly, cool temperatures activate the expression of a SUMO E3 ligase gene (*SIZ1*) in apple, which specifically stabilizes MYB transcription factors facilitating anthocyanin biosynthesis (Zhou et al., 2017). A flowering-time-independent pathway similar to this one is more consistent with our data.

Our study detected a cytonuclear interaction at the QTL FT.Cool.4 (Figure 4). At cool temperatures, the Danish cytoplasm eliminated the flowering time differences observed among F_2 nuclear combinations at warm temperature. Thus, at this QTL, the entire phenotypic effect of underlying flowering time variation was determined by strong epistasis between cytoplasm type and the nuclear genotype. This observation adds to a growing body of evidence for cytonuclear interactions affecting adaptive traits. Several recent studies have found evidence of cytonuclear interactions on phenotypic variation, such as flowering in maize and in *A. lyrata* (Leinonen et al., 2013; Tang et al., 2013), and cytonuclear incompatibilities appear in divergent eukaryote taxa from yeast (Chou, Hung, Lin, Lee, & Leu, 2010), to plants (Fishman & Willis, 2006; Sambatti, Ortiz-Barrientos, Baack, & Rieseberg, 2008) and animals (Gagnaire, Normandeau, & Bernatchez, 2012; Niehuis, Judson, & Gadau, 2008). Cytoplasmic genomes may serve as new sources of variation to accelerate evolutionary changes because they can modify the magnitude of some QTLs controlling trait variation, and thus gene networks (Roux et al., 2016; Soltani et al., 2016). Yet, despite their potential importance, the genetic

mechanisms underlying cytonuclear interactions remain obscure (Bock, Andrew, & Rieseberg, 2014; Budar & Roux, 2011; Roux et al., 2016; Soltani et al., 2016). A nuclear-encoded pentatricopeptide repeat (PPR) protein, POCO1, has been shown to regulate flowering time in *A. thaliana* via mitochondrial RNA editing (Emami & Kempken, 2019), suggesting one possible mechanism that could underlie the cytonuclear interaction we have observed.

This study provides the first published genetic map of *P. lanceolata*. The map contained 47 evenly spaced markers along six LGs, with an average marker spacing of 10.1 cM, which was ideal spacing to maximize the resolving power of our marker–QTL linkage experiment (Darvasi et al., 1993). Thus, the map provides insights about the genome of a nonmodel perennial plant species that has been and continues to be the subject of research in the areas of ecology and evolution (e.g., Halbritter, Billeter, Edwards, & Alexander, 2015; Levsen, Bergero, Charlesworth, & Wolff, 2016; Marshall et al., 2019; Ravenscroft et al., 2015; Wan, Fazlioglu, & Bonser, 2018; Watson-Lazowski et al., 2016) and agriculture (e.g., Gupta, 2017; Miglécz et al., 2015; Patton, Weisenberger, & Schortgen, 2018). Also, the study raises questions for future genetic research in *P. lanceolata*, such as examining further the genetic control of thermal plasticity. Some of our findings, including genomic regions of distorted transmission ratios and heterogeneity among genotypes in the number of ddRAD tags that could be recovered, suggest possible variation in genome structure and the possibility of incipient speciation, which warrant further exploration.

Finally, this QTL study suggests how some plants will be able to accommodate rising temperatures, associated with climate change. Climate change is expected to impose strong directional selection pressures on plant populations (Anderson et al., 2012; Bradshaw & Holzapfel, 2008; Davis & Shaw, 2001; Jump & Penuelas, 2005; Ravenscroft et al., 2015; Reusch & Wood, 2007). If populations of short-lived species are genetically variable, as we see for thermal plasticity in *P. lanceolata* and other species (Anderson, Lovin, et al., 2013), then populations may be more likely to survive under climatic warming. Flowering time and floral reflectance are both traits that help plants to reproduce at temperatures favourable for seed production. The former determines the window of reproduction and the latter is a partial thermoregulatory mechanism that functions within that window and can extend that window. Our QTL study suggests that thermal plasticity can help plants to respond because: (a) populations are genetically variable for plasticity in both traits; (b) one major gene (or one tight collection of genes) underlies the plasticity in floral reflectance, which should allow for more rapid evolutionary responses to climate change; and (c) plasticity for the two traits has the potential to evolve independently. Thermal plasticity characterizes the natural latitudinal and altitudinal variation in European populations of *P. lanceolata*. It is likely to influence how it and other species evolve over time, with the caveat that there are likely to be limits to any individual's ability to respond via phenotypic plasticity. Natural tolerance limits determined by genetic constraints and metabolic costs of extreme plasticity should prevent perpetually increasing plasticity. Where these limits are, however, is currently unclear.

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AUTHOR CONTRIBUTIONS

M.M.M., D.L.R. and E.P.L. conceived the experimental design; M.M.M. gathered phenotypic measures and conducted laboratory work; M.M.M., D.L.R. and E.P.L. performed analyses and wrote the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available. Genomic DNA sequence raw reads are available at NCBI: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA589951; Phenotypic data, R scripts, and associated input files are available at Dryad: https://doi.org/10.5061/dryad.fttdz08nq.

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REFERENCES

Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*, 739–745.

Ågren, J., Oakley, C. G., Lundemo, S., & Schemske, D. W. (2017). Adaptive divergence in flowering time among natural populations of *Arabidopsis thaliana*: Estimates of selection and QTL mapping. *Evolution*, *71*(3), 550–564.

Alonso-Blanco, C., & Méndez-Vigo, B. (2014). Genetic architecture of naturally occurring quantitative traits in plants: An updated synthesis. *Current Opinion in Plant Biology*, *18*, 37–43. https://doi.org/10.1016/j.pbi.2014.01.002

Alonso-Blanco, C., Mendez-Vigo, B., & Koornneef, M. (2004). From phenotypic to molecular polymorphisms involved in naturally occurring variation of plant development. *International Journal of Developmental Biology*, *49*, 717–732. https://doi.org/10.1387/ijdb.051994ca

Anderson, E. R., Lovin, M. E., Richter, S. J., & Lacey, E. P. (2013). Multiple *Plantago* species (Plantaginaceae) modify floral reflectance and color in response to thermal change. *American Journal of Botany*, *100*, 2485–2493.

Anderson, J. T., Inouye, D. W., McKinney, A. M., Colautti, R. I., & Mitchell-Olds, T. (2012). Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 3843–3852. https://doi.org/10.1098/rspb.2012.1051

Anderson, J. T., Lee, C., Rushworth, C. A., Colautti, R. I., & Mitchell-Olds, T. (2013). Genetic trade-offs and conditional neutrality contribute to local adaptation. *Molecular Ecology*, *22*, 699–708. https://doi.org/10.1111/j.1365-294X.2012.05522.x

Anderson, J. T., Willis, J. H., & Mitchell-Olds, T. (2011). Evolutionary genetics of plant adaptation. *Trends in Genetics: TIG*, *27*, 258–266. https://doi.org/10.1016/j.tig.2011.04.001

Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. Oxford, UK: Oxford University Press.

Auld, J. R., Agrawal, A. A., & Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, *277*, 503–511. https://doi.org/10.1098/rspb.2009.1355

Bock, D. G., Andrew, R. L., & Rieseberg, L. H. (2014). On the adaptive value of cytoplasmic genomes in plants. *Molecular Ecology*, *23*, 4899–4911. https://doi.org/10.1111/mec.12920

Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M., … Roux, F. (2010). Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS Genetics*, *6*, e1000940. https://doi.org/10.1371/journal.pgen.1000940

Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advanced Genetics*, *13*, 115–155.

Bradshaw, W. E., & Holzapfel, C. M. (2008). Genetic response to rapid climate change: It's seasonal timing that matters. *Molecular Ecology*, *17*, 157–166. https://doi.org/10.1111/j.1365-294X.2007.03509.x

Broman, K. W., Wu, H., Sen, S., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, *19*, 889–890. https://doi.org/10.1093/bioinformatics/btg112

Budar, F., & Roux, F. (2011). The role of organelle genomes in plant adaptation: Time to get to work!. *Plant Signaling and Behavior*, *6*, 635–639. https://doi.org/10.4161/psb.6.5.14524

Burgarella, C., Chantret, N., Gay, L., Prosperi, J., Bonhomme, M., Tiffin, P., … Ronfort, J. (2016). Adaptation to climate through flowering phenology: A case study in *Medicago truncatula*. *Molecular Ecology*, *25*, 3397–3415.

Case, A. L., Lacey, E. P., & Hopkins, R. G. (1996). Parental effects in *Plantago lanceolata* L. 2. Manipulation of grandparental temperature and parental flowering time. *Heredity*, *76*, 287–295.

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes, Genetics*, *1*, 171–182.

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, *22*, 3124–3140. https://doi.org/10.1111/mec.12354

Cavers, P. B., Bassett, I. J., & Crompton, C. W. (1980). The biology of Canadian Weeds.: 47. *Plantago lanceolata* L. *Canadian Journal of Plant Science*, *60*, 1269–1282.

Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biology*, *8*, e1000357.

Chou, J.-Y., Hung, Y.-S., Lin, K.-H., Lee, H.-Y., & Leu, J.-Y. (2010). Multiple molecular mechanisms cause reproductive isolation between three yeast species. *PLoS Biology*, *8*, e1000432.

Churchill, G. A., & Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, *138*, 963–971.

Clench, H. K. (1966). Behavioral thermoregulation in butterflies. *Ecology*, *47*, 1021–1034. https://doi.org/10.2307/1935649

Colosimo, P. F., Hosemann, K. E., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., … Kingsley, D. M. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*, *307*, 1928–1933. https://doi.org/10.1126/science.1107239

Crick, H. Q. P., Dudley, C., Glue, D. E., & Thomson, D. L. (1997). Long-term trends towards earlier egg-laying by UK birds. *Nature*, *388*, 526.

Crispo, E. (2007). The Baldwin effect and genetic assimilation: Revisiting two mechanisms of evolutionary change ediated by phenotypic plasticity. *Evolution*, *61*, 2469–2479. https://doi.org/10.1111/j.1558-5646.2007.00203.x

Darvasi, A., Weinreb, A., Minke, V., Weller, J. I., & Soller, M. (1993). Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. *Genetics*, *134*, 943–951.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, *292*, 673–679. https://doi.org/10.1126/science.292.5517.673

Davis, M. B., Shaw, R. G., & Etterson, J. R. (2005). Evolutionary responses to changing climate. *Ecology*, *86*, 1704–1714. https://doi.org/10.1890/03-0788

Des Marais, D. L., Hernandez, K. M., & Juenger, T. E. (2013). Genotype-by-environment interaction and plasticity: Exploring genomic responses of plants to the abiotic environment. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 5–29. https://doi.org/10.1146/annurev-ecolsys-110512-135806

Dittmar, E. L., Oakley, C. G., Ågren, J., & Schemske, D. W. (2014). Flowering time QTL in natural populations of *Arabidopsis thaliana* and implications for their adaptive value. *Molecular Ecology*, *23*, 4291–4303.

Dittmar, E. L., Oakley, C. G., Conner, J. K., Gould, B. A., & Schemske, D. W. (2016). Factors influencing the effect size distribution of adaptive substitutions. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20153065. https://doi.org/10.1098/rspb.2015.3065

Ehleringer, J., & Forseth, I. (1980). Solar tracking by plants. *Science*, *210*, 1094–1098. https://doi.org/10.1126/science.210.4474.1094

El-Soda, M., Malosetti, M., Zwaan, B. J., Koornneef, M., & Aarts, M. G. M. (2014). Genotype× environment interaction QTL mapping in plants: Lessons from Arabidopsis. *Trends in Plant Science*, *19*, 390–398. https://doi.org/10.1016/j.tplants.2014.01.001

Emami, H., & Kempken, F. (2019). PRECOCIOUS1 (POCO1), a mitochondrial pentatricopeptide repeat (PPR) protein affects flowering time in *Arabidopsis thaliana*. *The Plant Journal*, *100*, 265–278.

Fisher, R. A. (1919). XV.—The correlation between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh*, *52*, 399–433. https://doi.org/10.1017/S0080456800012163

Fisher, R. A. (1930). *The genetical theory of natural selection: A complete variorum edition*. Oxford, UK: Oxford University Press.

Fishman, L., & Willis, J. H. (2006). A cytonuclear incompatibility causes anther sterility in *Mimulus* hybrids. *Evolution*, *60*, 1372–1381.

Fitter, A. H., & Fitter, R. S. R. (2002). Rapid changes in flowering time in British plants. *Science*, *296*, 1689–1691. https://doi.org/10.1126/science.1071617

Frankel, N., Wang, S., & Stern, D. L. (2012). Conserved regulatory architecture underlies parallel genetic changes and convergent phenotypic evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 20975–20979. https://doi.org/10.1073/pnas.1207715109

Gagnaire, P.-A., Normandeau, E., & Bernatchez, L. (2012). Comparative genomics reveals adaptive protein evolution and a possible cytonuclear incompatibility between European and American eels. *Molecular Biology and Evolution*, *29*(10), 2909–2919.

García-Gil, M. R., Mikkonen, M., & Savolainen, O. (2003). Nucleotide diversity at two phytochrome loci along a latitudinal cline in *Pinus sylvestris*. *Molecular Ecology*, *12*, 1195–1206. https://doi.org/10.1046/j.1365-294X.2003.01826.x

Gaston, K. J., & Chown, S. L. (1999). Elevation and climatic tolerance: A test using dung beetles. *Oikos*, *86*, 584–590. https://doi.org/10.2307/3546663

Gerats, A. G. M., Farcy, E., Wallroth, M., Groot, S. P. C., & Schram, A. (1984). Control of anthocyanin synthesis in *Petunia hybrida* by multiple allelic series of the genes An1 and An2. *Genetics*, *106*, 501–508.

Gerken, A. R., Eller, O. C., Hahn, D. A., & Morgan, T. J. (2015). Constraints, independence, and evolution of thermal plasticity: Probing genetic architecture of long-and short-term thermal acclimation. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 4399–4404. https://doi.org/10.1073/pnas.1503456112

Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J., & Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's

hypothesis revisited. *Integrative and Comparative Biology*, *46*, 5–17. https://doi.org/10.1093/icb/icj003

Gitelson, A. A., Merzlyak, M. N., & Chivkunova, O. B. (2001). Optical properties and nondestructive estimation of anthocyanin content in plant leaves. *Photochemistry and Photobiology*, *74*, 38–45. https://doi.org/10.1562/0031-8655(2001)074<0038:OPANEO>2.0.CO;2

Grillo, M. A., Li, C., Hammond, M., Wang, L., & Schemske, D. W. (2013). Genetic architecture of flowering time differentiation between locally adapted populations of *Arabidopsis thaliana*. *New Phytologist*, *197*, 1321–1331.

Gu, K.-D., Wang, C.-K., Hu, D.-G., & Hao, Y.-J. (2019). How do anthocyanins paint our horticultural products? *Scientia Horticulturae*, *249*, 257–262. https://doi.org/10.1016/j.scienta.2019.01.034

Gupta, R. (2017). Agrotechnology of medicinal plants. In R. O. B. Wijesekera (Ed.), *The medicinal plant industry* (pp. 43–58). London, UK: Routledge.

Halbritter, A. H., Billeter, R., Edwards, P. J., & Alexander, J. M. (2015). Local adaptation at range edges: Comparing elevation and latitudinal gradients. *Journal of Evolutionary Biology*, *28*, 1849–1860. https://doi.org/10.1111/jeb.12701

Hall, M. C., Basten, C. J., & Willis, J. H. (2006). Pleiotropic quantitative trait loci contribute to population divergence in traits associated with life-history variation in *Mimulus guttatus*. *Genetics*, *172*, 1829–1844.

Hall, M. C., & Willis, J. H. (2007). Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution*, *60*, 2466–2477. https://doi.org/10.1111/j.0014-3820.2006.tb01882.x

Hazel, J. R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annual Review of Physiology*, *57*, 19–42.

Hoffmann, A. A., Sørensen, J. G., & Loeschcke, V. (2003). Adaptation of Drosophila to temperature extremes: Bringing together quantitative and molecular approaches. *Journal of Thermal Biology*, *28*, 175–216. https://doi.org/10.1016/S0306-4565(02)00057-8

Huey, R. B. (1991). Physiological consequences of habitat selection. *American Naturalist*, *137*, 91–115. https://doi.org/10.1086/285141

Huey, R. B., & Bennett, A. F. (1990). Physiological adjustments to fluctuating thermal environments: An ecological and evolutionary perspective. In R. I. Morimoto, A. Tissieres, & C. Georgopoulos (Eds.), *Stress proteins in biology and medicine* (pp. 37–59). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Huey, R. B., & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist*, *19*, 357–366. https://doi.org/10.1093/icb/19.1.357

Johansson, M., & Staiger, D. (2014). Time to flower: Interplay between photoperiod and the circadian clock. *Journal of Experimental Botany*, *66*, 719–730. https://doi.org/10.1093/jxb/eru441

Jump, A. S., & Penuelas, J. (2005). Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters*, *8*, 1010–1020. https://doi.org/10.1111/j.1461-0248.2005.00796.x

Kitano, H. (2004). Biological robustness. *Nature Reviews Genetics*, *5*, 826. https://doi.org/10.1038/nrg1471

Komsta, L., & Novomestky, F. (2015) *Moments: Moments, cumulants, skewness, kurtosis and related tests*. Retrieved from http://cran.r-project.org/package=moments

Kosambi, D. D. (1943). The estimation of map distances from recombination values. *Annals of Eugenics*, *12*, 172–175. https://doi.org/10.1111/j.1469-1809.1943.tb02321.x

Kudo, G. (1995). Ecological significance of flower heliotropism in the spring ephemeral *Adonis ramosa* (Ranunculaceae). *Oikos*, *72*, 14–20. https://doi.org/10.2307/3546032

Lacey, E. P. (1988). Latitudinal variation in reproductive timing of a short-lived monocarp, *Daucus carota* (Apiaceae). *Ecology*, *69*, 220–232. https://doi.org/10.2307/1943178

Lacey, E. P. (1996). Parental effects in Plantago lanceolata L.1. A growth chamber experiment to examine pre- and postzygotic temperature effects. *Evolution*, *50*, 865–878.

Lacey, E. P., & Herr, D. (2000). Parental effects in *Plantago lanceolata* L. III. Measuring parental temperature effects in the field. *Evolution*, *54*, 1207–1217. https://doi.org/10.1111/j.0014-3820.2000.tb00555.x

Lacey, E. P., & Herr, D. (2005). Phenotypic plasticity, parental effects and parental care in plants? I. An examination of spike reflectance in *Plantago lanceolata* (Plantaginaceae). *American Journal of Botany*, *92*, 920–930.

Lacey, E. P., Lovin, M. E., & Richter, S. J. (2012). Fitness effects of floral plasticity and thermoregulation in a thermally changing environment. *American Naturalist*, *180*, 342. https://doi.org/10.1086/666987

Lacey, E. P., Lovin, M. E., Richter, S. J., & Herington, D. A. (2010). Floral reflectance, color, and thermoregulation: What really explains geographic variation in thermal acclimation ability of ectotherms? *The American Naturalist*, *175*, 335–349. https://doi.org/10.1086/650442

Lamichhaney, S., Han, F., Berglund, J., Wang, C., Almén, M. S., Webster, M. T., … Andersson, L. (2016). A beak size locus in Darwin's finches facilitated character displacement during a drought. *Science*, *352*, 470–474. https://doi.org/10.1126/science.aad8786

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, *22*, 1435–1446. https://doi.org/10.1111/j.1420-9101.2009.01754.x

Lange, K., & Boehnke, M. (1982). How many polymorphic marker genes will it take to span the human genome? *American Journal of Human Genetics*, *34*, 842.

Laugen, A. T., Laurila, A., Räsänen, K., & Merilä, J. (2003) Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates – Evidence for local adaptation. *Journal of Evolutionary Biology*, *16*, 996–1005. https://doi.org/10.1046/j.1420-9101.2003.00560.x

Lee, Y. W., Gould, B. A., & Stinchcombe, J. R. (2014). Identifying the genes underlying quantitative traits: A rationale for the QTN programme. *AoB PLANTS*, *6*, plu004. https://doi.org/10.1093/aobpla/plu004

Legris, M., Nieto, C., Sellaro, R., Prat, S., & Casal, J. J. (2017). Perception and signalling of light and temperature cues in plants. *The Plant Journal*, *90*, 683–697. https://doi.org/10.1111/tpj.13467

Leinonen, P. H., Remington, D. L., Leppälä, J., Savolainen, O., Leppelae, J., & Savolainen, O. (2013). Genetic basis of local adaptation and flowering time variation in *Arabidopsis lyrata*. *Molecular Ecology*, *22*, 709–723.

Levsen, N., Bergero, R., Charlesworth, D., & Wolff, K. (2016). Frequent, geographically structured heteroplasmy in the mitochondria of a flowering plant, ribwort plantain (*Plantago lanceolata*). *Heredity*, *117*, 1. https://doi.org/10.1038/hdy.2016.15

Li, L., Ban, Z.-J., Li, X.-H., Wu, M.-Y., Wang, A.-L., Jiang, Y.-Q., & Jiang, Y.-H. (2012a). Differential expression of anthocyanin biosynthetic genes and transcription factor PcMYB10 in pears (*Pyrus communis* L.). *PLoS ONE*, *7*, e46070. https://doi.org/10.1371/journal.pone.0046070

Li, Y.-Y., Mao, K., Zhao, C., Zhao, X.-Y., Zhang, H.-L., Shu, H.-R., & Hao, Y.-J. (2012b). MdCOP1 ubiquitin E3 ligases interact with MdMYB1 to regulate light-induced anthocyanin biosynthesis and red fruit coloration in apple. *Plant Physiology*, *160*, 1011–1022. https://doi.org/10.1104/pp.112.199703

Liefting, M., Hoffmann, A. A., & Ellers, J. (2009). Plasticity versus environmental canalization: Population differences in thermal responses along a latitudinal gradient in *Drosophila serrata*. *Evolution*, *63*, 1954–1963.

Linnen, C. R., Poh, Y.-P., Peterson, B. K., Barrett, R. D. H., Larson, J. G., Jensen, J. D., & Hoekstra, H. E. (2013). Adaptive evolution of multiple traits through multiple mutations at a single gene. *Science*, *339*, 1312–1316. https://doi.org/10.1126/science.1233213

Mahmud, I., & Kramer, H. H. (1951). Segregation for yield, height, and maturity following a soybean cross. *Agronomy Journal*, *43*, 605–609.

Margarido, G. R. A., de Souza, A. P., & Garcia, A. A. F. (2007). OneMap: Software for genetic mapping in outcrossing species. *Hereditas*, *144*, 78–79. https://doi.org/10.1111/j.2007.0018-0661.02000.x

Marmur, J., & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature.

Journal of Molecular Biology, *5*, 109–118. https://doi.org/10.1016/S0022-2836(62)80066-7

Marshall, M. M. (2017). *The genetics of thermal plasticity in* Plantago lanceolata. Greensboro, NC: University of North Carolina at Greensboro. Retrieved from https://libres.uncg.edu/ir/uncg/f/Marshall_uncg_0154D_12304.pdf

Marshall, M. M., Batten, L. C., Remington, D. L., & Lacey, E. P. (2019) Natural selection contributes to geographic patterns of thermal plasticity in *Plantago lanceolata*. *Ecology and Evolution*, *9*(5), 2945–2963. https://doi.org/10.1002/ece3.4977

Marshall, M. M., Remington, D. L., & Lacey, E. P. (2019a). *Supporting gDNA sequence data: Two reproductive traits show contrasting genetic architectures in* Plantago lanceolata. Retrieved from https://www.ncbi.nlm.nih.gov/bioproject/PRJNA589951

Marshall, M. M., Remington, D. L., & Lacey, E. P. (2019b). *Supporting phenotypic data, R scripts, and R input files: Two reproductive traits show contrasting genetic architectures in* Plantago lanceolata. https://doi.org/10.5061/dryad.fttdz08nq

Martin, A., & Orgogozo, V. (2013). The loci of repeated evolution: A catalog of genetic hotspots of phenotypic variation. *Evolution*, *67*, 1235–1250. https://doi.org/10.1111/evo.12081

McGregor, A. P., Orgogozo, V., Delon, I., Zanet, J., Srinivasan, D. G., Payre, F., & Stern, D. L. (2007). Morphological evolution through multiple cis-regulatory mutations at a single gene. *Nature*, *448*, 587. https://doi.org/10.1038/nature05988

Méndez-Vigo, B., Savic, M., Ausín, I., Ramiro, M., Martín, B., Picó, F. X., & Alonso-Blanco, C. (2016). Environmental and genetic interactions reveal FLOWERING LOCUS C as a modulator of the natural variation for the plasticity of flowering in Arabidopsis. *Plant, Cell & Environment*, *39*, 282–294.

Merzlyak, M. N., Chivkunova, O. B., Solovchenko, A. E., & Naqvi, K. R. (2008). Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. *Journal of Experimental Botany*, *59*, 3903–3911. https://doi.org/10.1093/jxb/ern230

Miglécz, T., Valkó, O., Török, P., Deák, B., Kelemen, A., Donkó, Á., … Tóthmérész, B. (2015). Establishment of three cover crop mixtures in vineyards. *Scientia Horticulturae*, *197*, 117–123. https://doi.org/10.1016/j.scienta.2015.09.017

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., … van Kleunen, M. (2010). Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, *15*, 684–692. https://doi.org/10.1016/j.tplants.2010.09.008

Niehuis, O., Judson, A. K., & Gadau, J. (2008). Cytonuclear genic incompatibilities cause increased mortality in male F2 hybrids of *Nasonia girault*i and *N. vitripennis*. *Genetics*, *178*, 413–426.

Olsson, K., & Ågren, J. (2002). Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb *Lythrum salicaria*. *Journal of Evolutionary Biology*, *15*, 983–996.

Orr, H. A. (1998). The population genetics of adaptation: The distribution of factors fixed during adaptive evolution. *Evolution*, 935–949. https://doi.org/10.1111/j.1558-5646.1998.tb01823.x

Orr, H. A. (2005). The genetic theory of adaptation: A brief history. *Nature Reviews Genetics*, *6*, 119–127. https://doi.org/10.1038/nrg1523

Patton, A. J., Weisenberger, D. V., &Schortgen, G. P. (2018). 2, 4-D–resistant buckhorn plantain (*Plantago lanceolata*) in managed turf. *Weed Technology*, *32*, 182–189.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, *7*, e37135. https://doi.org/10.1371/journal.pone.0037135

Primack, R. B., & Antonovics, J. (1981). Experimental ecological genetics in Plantago. V. Components of seed yield in the ribwort plantain *Plantago lanceolata* L. *Evolution*, *35*, 1069–1079. https://doi.org/10.2307/2408121

Qiu, Y., Li, M., Jean, R., Moore, C. M., & Chen, M. (2019). Daytime temperature is sensed by phytochrome B in *Arabidopsis* through a transcriptional activator HEMERA. *Nature Communications*, *10*, 140. https://doi.org/10.1038/s41467-018-08059-z

Quilot-Turion, B., Leppälä, J., Leinonen, P. H., Waldmann, P., Savolainen, O., & Kuittinen, H. (2013). Genetic changes in flowering and morphology in response to adaptation to a high-latitude environment in *Arabidopsis lyrata*. *Annals of Botany*, *111*, 957–968. https://doi.org/10.1093/aob/mct055

R Development Core Team, (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation Statistical Computing. Retrieved from http://www.r-project.org

Ravenscroft, C. H., Whitlock, R., & Fridley, J. D. (2015). Rapid genetic divergence in response to 15 years of simulated climate change. *Global Change Biology*, *21*, 4165–4176.

Remington, D. L. (2015). Alleles versus mutations: Understanding the evolution of genetic architecture requires a molecular perspective on allelic origins. *Evolution*, *69*, 3025–3038. https://doi.org/10.1111/evo.12775

Remington, D. L., Leinonen, P. H., Leppälä, J., & Savolainen, O. (2013). Complex genetic effects on early vegetative development shape resource allocation differences between *Arabidopsis lyrata* populations. *Genetics*, *195*, 1087–1102.

Remington, D. L., Whetten, R. W., Liu, B.-H., & O'malley, D. M. (1999). Construction of an AFLP genetic map with nearly complete genome coverage in *Pinus taeda*. *Theoretical and Applied Genetics*, *98*, 1279–1292. https://doi.org/10.1007/s001220051194

Reusch, T. B. H., & Wood, T. E. (2007). Molecular ecology of global change. *Molecular Ecology*, *16*, 3973–3992. https://doi.org/10.1111/j.1365-294X.2007.03454.x

Robertson, A. (1967). The nature of quantitative genetic variation. In A. Brink (Ed.), *Heritage From Mendel* 265–280. Madison, WI: University of Wisconsin-Madison.

Rockman, M. V. (2012). The QTN program and the alleles that matter for evolution: All that's gold does not glitter. *Evolution; International Journal of Organic Evolution*, *66*, 1–17. https://doi.org/10.1111/j.1558-5646.2011.01486.x

Ross, M. D. (1973). Inheritance of self-incompatibility in *Plantago lanceolata*. *Heredity*, *30*, 169–176. https://doi.org/10.1038/hdy.1973.19

Roux, F., Mary-Huard, T., Barillot, E., Wenes, E., Botran, L., Durand, S., … Budar, F. (2016). Cytonuclear interactions affect adaptive traits of the annual plant Arabidopsis thaliana in the field. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 3687-3692.

Royer, D. L., Meyerson, L. A., Robertson, K. M., & Adams, J. M. (2009). Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. *PLoS ONE*, *4*, e7653. https://doi.org/10.1371/journal.pone.0007653

Sambatti, J., Ortiz-Barrientos, D., Baack, E. J., & Rieseberg, L. H. (2008). Ecological selection maintains cytonuclear incompatibilities in hybridizing sunflowers. *Ecology Letters*, *11*, 1082–1091. https://doi.org/10.1111/j.1461-0248.2008.01224.x

Schlichting, C. D. (2008). Hidden reaction norms, cryptic genetic variation, and evolvability. *Annals of the New York Academy of Sciences*, *1133*, 187–203. https://doi.org/10.1196/annals.1438.010

Schmitt, J., Niles, J., & Wulff, R. D. (1992). Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. *The American Naturalist*, 451–466. https://doi.org/10.1086/285338

Shapiro, M. D., Marks, M. E., Peichel, C. L., Blackman, B. K., Nereng, K. S., Jónsson, B., … Kingsley, D. M. (2004). Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature*, *428*, 717. https://doi.org/10.1038/nature02415

Simpson, G. G. (1953). The Baldwin effect. *Evolution*, *7*, 110–117. https://doi.org/10.1111/j.1558-5646.1953.tb00069.x

Snyder, W. E. (1948). Mechanism of the photoperiodic response of *Plantago lanceolata* L., a long-day plant. *American Journal of Botany*, 520–525. https://doi.org/10.1002/j.1537-2197.1948.tb08115.x

Soltani, A., Kumar, A., Mergoum, M., Pirseyedi, S. M., Hegstad, J. B., Mazaheri, M., & Kianian, S. F. (2016). Novel nuclear-cytoplasmic interaction in wheat (*Triticum aestivum*) induces vigorous plants. *Functional and Integrative Genomics*, *16*, 171–182. https://doi.org/10.1007/s10142-016-0475-2

Somero, G. N. (1995). Proteins and temperature. *Annual Review of Physiology*, *57*, 43–68. https://doi.org/10.1146/annurev.ph.57.030195.000355

Song, K., Kim, H. C., Shin, S., Kim, K.-H., Moon, J.-C., Kim, J. Y., & Lee, B.-M. (2017). Transcriptome analysis of flowering time genes under drought stress in maize leaves. *Frontiers in Plant Science*, *8*, 267. https://doi.org/10.3389/fpls.2017.00267

Song, Y. H., Ito, S., & Imaizumi, T. (2013). Flowering time regulation: Photoperiod-and temperature-sensing in leaves. *Trends in Plant Science*, *18*, 575–583. https://doi.org/10.1016/j.tplants.2013.05.003

Stiles, E. A., Cech, N. B., Dee, S. M., & Lacey, E. P. (2007). Temperature-sensitive anthocyanin production in flowers of *Plantago lanceolata*. *Physiologia Plantarum*, *129*, 756–765. https://doi.org/10.1111/j.1399-3054.2007.00855.x

Supple, M. A., Hines, H. M., Dasmahapatra, K. K., Lewis, J. J., Nielsen, D. M., Lavoie, C., … Counterman, B. A. (2013). Genomic architecture of adaptive color pattern divergence and convergence in *Heliconius butterflies*. *Genome Research*, *23*, 1248–1257. https://doi.org/10.1101/gr.150615.112

Tang, Z., Yang, Z., Hu, Z., Zhang, D., Lu, X., Jia, B., … Xu, C. (2013). Cytonuclear epistatic quantitative trait locus mapping for plant height and ear height in maize. *Molecular Breeding*, *31*, 1–14. https://doi.org/10.1007/s11032-012-9762-3

van Berkum, F. H. (1988). Latitudinal patterns of the thermal sensitivity of sprint speed in lizards. *The American Naturalist*, *132*, 327–343. https://doi.org/10.1086/284856

Van Damme, J. M. M. (1983). Gynodioecy in *Plantago lanceolata* L. II. Inheritance of three male sterility types. *Heredity*, *50*, 253–273. https://doi.org/10.1038/hdy.1983.28

Van Hinsberg, A., & Van Tienderen, P. (1997). Variation in growth form in relation to spectral light quality (red/far-red ratio) in *Plantago lanceolata* L. in sun and shade populations. *Oecologia*, *111*, 452–459. https://doi.org/10.1007/s004420050258

Van Tienderen, P. H. (1990). Morphological variation in *Plantago lanceolata*: Limits of plasticity. *Evolutionary Trends in Plants*, *4*, 35–43.

Van Tienderen, P. H. (1992). Variation in a population of *Plantago lanceolata* along a topographical gradient. *Oikos*, *64*, 560–572. https://doi.org/10.2307/3545176

van Tienderen, P. H., & van der Toorn, J. (1991a). Genetic differentiation between populations of *Plantago lanceolata*. II. Phenotypic selection in a transplant experiment in three contrasting habitats. *The Journal of Ecology*, *79*, 43–59. https://doi.org/10.2307/2260783

van Tienderen, P. H., & van der Toorn, J. (1991b). Genetic differentiation between populations of *Plantago lanceolata*. I. Local adaptation in three contrasting habitats. *The Journal of Ecology*, *79*, 27–42. https://doi.org/10.2307/2260782

Vedder, O., Bouwhuis, S., & Sheldon, B. C. (2013). Quantitative assessment of the importance of phenotypic plasticity in adaptation to climate change in wild bird populations. *PLoS Biology*, *11*, e1001605. https://doi.org/10.1371/journal.pbio.1001605

Visser, M. E., & Holleman, L. J. M. (2001). Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*, 289–294.

Waldmann, P., Garcia-Gil, M. R., & Sillanpää, M. J. (2005). Comparing Bayesian estimates of genetic differentiation of molecular markers and quantitative traits: An application to *Pinus sylvestris*. *Heredity*, *94*, 623–629. https://doi.org/10.1038/sj.hdy.6800672

Wan, J. S. H., Fazlioglu, F., & Bonser, S. P. (2018). Loss of plasticity in life-history strategy associated with secondary invasion into stressful environments in invasive narrowleaf plantain (*Plantago lanceolata* L.). *Austral Ecology*, *43*, 752–762.

Watson-Lazowski, A., Lin, Y., Miglietta, F., Edwards, R. J., Chapman, M. A., & Taylor, G. (2016). Plant adaptation or acclimation to rising CO_2? Insight from first multigenerational RNA-Seq transcriptome. *Global Change Biology*, *22*, 3760–3773.

Webster, M. D., & Weathers, W. W. (1990). Heat produced as a by-product of foraging activity contributes to thermoregulation by verdins. *Auriparus Flaviceps*. *Physiological Zoology*, 777–794.

Wellenreuther, M., & Hansson, B. (2016). Detecting polygenic evolution: Problems, pitfalls, and promises. *Trends in Genetics*, *32*, 155–164. https://doi.org/10.1016/j.tig.2015.12.004

Wolff, K. (1987). Genetic analysis of ecological relevant morphological variability in *Plantago lanceolata* L. II: Localisation and organisation of quantitative trait loci. *Theoretical and Applied Genetics*, *73*(6), 903–914.

Wolff, K., & Van Delden, W. (1987). Genetic analysis of ecological relevant morphological variability in *Plantago lanceolata* L. I Population characteristics. *Heredity*, *58*, 183–192. https://doi.org/10.1038/hdy.1987.32

Wu, J., Wang, W., Xu, P., Pan, J., Zhang, T., Li, Y., … Lian, H. (2018). phyB Interacts with BES1 to regulate brassinosteroid signaling in Arabidopsis. *Plant and Cell Physiology*, *60*, 353–366.

Wu, R., Ma, C.-X., Painter, I., & Zeng, Z.-B. (2002). Simultaneous maximum likelihood estimation of linkage and linkage phases in outcrossing species. *Theoretical Population Biology*, *61*, 349–363. https://doi.org/10.1006/tpbi.2002.1577

Yadav, A., Dhole, K., & Sinha, H. (2016). Genetic regulation of phenotypic plasticity and canalisation in yeast growth. *PLoS ONE*, *11*, e0162326. https://doi.org/10.1371/journal.pone.0162326

Yeaman, S., & Whitlock, M. C. (2011). The genetic architecture of adaptation under migration–selection balance. *Evolution: International Journal of Organic Evolution*, *65*, 1897–1911. https://doi.org/10.1111/j.1558-5646.2011.01269.x

Zhang, K. M., Wang, J. W., Guo, M. L., Du, W. L., Wu, R. H., & Wang, X. (2016). Short-day signals are crucial for the induction of anthocyanin biosynthesis in *Begonia semperflorens* under low temperature condition. *Journal of Plant Physiology*, *204*, 1–7. https://doi.org/10.1016/j.jplph.2016.06.021

Zhang, Q., Hao, R., Xu, Z., Yang, W., Wang, J., Cheng, T., … Zhang, Q. (2017). Isolation and functional characterization of a R2R3-MYB regulator of *Prunus mume* anthocyanin biosynthetic pathway. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *131*, 417–429. https://doi.org/10.1007/s11240-017-1294-4

Zhou, H., Lin-Wang, K., Wang, F., Espley, R. V., Ren, F., Zhao, J., … Han, Y. (2019). Activator-type R2R3-MYB genes induce a repressor-type R2R3-MYB gene to balance anthocyanin and proanthocyanidin accumulation. *New Phytologist*, *221*, 1919–1934. https://doi.org/10.1111/nph.15486

Zhou, L.-J., Mao, K., Qiao, Y., Jiang, H., Li, Y.-Y., & Hao, Y.-J. (2017). Functional identification of MdPIF1 as a phytochrome interacting factor in apple. *Plant Physiology and Biochemistry*, *119*, 178–188. https://doi.org/10.1016/j.plaphy.2017.08.027

SUPPORTING INFORMATION

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

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