International Journal of Plant Developmental Biology ©2010 Global Science Books

Genetic and Environmental Variation Affect the Ontogeny of Reproductive Traits in *Arabidopsis thaliana*

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ABSTRACT

Despite the wealth of molecular information about inflorescence development in the model plant *Arabidopsis thaliana*, we know much less about how traits involved in reproduction vary and covary at a phenotypic level, even though phenotypic variation and covariation are the substrates of natural selection and subsequent evolution. If we are to understand *A. thaliana*'s microevolutionary dynamics to the same extent that we understand its molecular genetics, then we must first flesh out and describe this (co)-variation. We characterized the covariation of reproductive traits in *A. thaliana*, utilizing multiple natural genotypes to assess whether such covariation is genetically variable. We subjected plants to naturally relevant variation in apical meristem damage and nutrient levels to explore the degree to which the relationships among traits are plastic. We found that inflorescence ontogeny (as inferred from the relationships among reproductive traits) is altered in apically damaged plants, and that variation in nutrient levels affects ontogeny as well. We also found that genetically clustered groups of plants qualitatively differ in the relationships among traits. These findings are discussed in terms of constraints on selection and of possible selection pressures for different inflorescence ontogenies in this species.

Keywords: apical meristem damage, allometry, *Arabidopsis thaliana*, constraint, fitness components, nutrient levels, path analysis, ontogeny, phenotypic plasticity

INTRODUCTION

Arabidopsis thaliana is well known as one of the best-studied organisms on a molecular genetic level, facilitated by the large community of researchers, its ease of growth, and the suite of molecular tools developed around it (Pang and Meyerowitz 1987; Meyerowitz 2001; Simpson and Dean 2002; Mitchell-Olds and Schmitt 2006; Leonelli 2007). Despite a history as a research organism tracing back over sixty years (summarized in Griffing and Scholl 1991; Meyerowitz 2001; Pigliucci 2003a; Leonelli 2007), the overall body of work on *A. thaliana* is lopsided towards molecular genetic studies of laboratory-reared strains (with the notable exception of phenotypic plasticity studies, e.g., Westerman and Lawrence 1970; Pigliucci and Schlichting 1995; Callahan and Pigliucci 2002; Tonsor and Scheiner 2007).

Only relatively recently has *A. thaliana*'s entire phenotype been studied in an ecological context and in an integrated fashion (for some examples, see Pigliucci and Schlichting 1998; Camara and Pigliucci 1999; Scheiner *et al.* 2000; Pigliucci 2003a; Pigliucci and Kolodynska 2006; Tonsor and Scheiner 2007). This is significant for several reasons. First, there are a variety of questions in ecology and evolution that can be addressed using *A. thaliana* (e.g., competition: Weltzin *et al.* 2003; life history evolution: Donohue *et al.* 2005; phenotypic plasticity: Banta *et al.* 2007; maternal effects: Bossdorf *et al.* 2009; quantitative epigenetics: Bossdorf *et al.* 2007). *A. thaliana* presents us with some of the same advantages for use in ecology and evolution studies as in molecular genetic studies. In fact, despite often being portrayed as a laboratory organism, *A. thaliana* actually has extensive natural phenotypic and life history variation and a relatively wide niche breadth (Donohue 2002; Pigliucci 2003a; Mitchell-Olds and Schmitt 2006). Furthermore, while it is appreciated that genetic networks can be extremely complex, it is less appreciated that the same holds true for phenotypic complexity (Wagner 2001; Pigliucci 2003b; West-Eberhard 2003a; Pigliucci and Preston 2004; Klingenberg 2008). *A. thaliana* is an efficient vehicle for mapping both levels of complexity.

Second, because of the wealth of molecular information about *A. thaliana* and the relative ease of collection of its phenotypic information, there is a great potential for cross-talk between molecular biology, ecology and evolutionary biology that can be mediated by the common use of *A. thaliana* as a cross-disciplinary model system (e.g., Caicedo *et al.* 2004; Wilczek *et al.* 2009).

Third, one of the implicit reasons to study a model organism is to be able to transfer the information to a wider group of species that are less practical to work with (e.g., Mitchell-Olds 2001; Zhang *et al.* 2004). This transfer of information should involve phenotypic, as well as molecular, studies, if *A. thaliana*'s potential as a model system is to be fully realized.

Multivariate phenotypes are traditionally studied in four ways (where the latter three are nested within the first): (1) summarizing trait variation and covariation using a matrix of variances and pairwise covariances (i.e., the variance-covariance matrix), and evaluating/comparing its various properties and lability (Steppan *et al.* 2002; McGuigan 2006; Blows 2007); (2) using the information about variances and covariances to regress all of the traits on one particular trait of interest, usually a measure of fitness (multiple linear regression analysis; Sokal and Rohlf 1995; Legendre 1998; Blows 2007); (3) using the variance-covariance matrix (or its standardized counterpart) to "collapse" the hyper-dimensional relationships among traits to a smaller number of axes of variation (principal components) that are more easily summarized and evaluated/compared (Jolliffe 2002; see Mezey and Houle 2003 for a particularly relevant example); and (4) using the variance-covariance matrix to cluster

Received: 12 May, 2009. Accepted: 21 April, 2010.

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observations (traits) based on trait similarity (discriminant analysis – Friedman 1989 – and cluster analysis – Aldenderfer and Blashfield 1984). (With some data sets, geometric morphometric methods are also commonly applied; Zelditch *et al.* 2004). There are also new methods being developed for combining information about multivariate phenotypes with the specific loci controlling their variation (Li *et al.* 2006; Brock *et al.* 2009; Kelly 2009; Remington 2009).

The traditional methods are limited by the fact that they do not account for the inherent directionality of causal relationships among traits. This distinction is best illustrated by the difference between correlation (bidirectional, or causally agnostic) and regression (unidirectional, implying a particular pattern of causation) analyses (Sokal and Rohlf 1995). Standard methods also do not allow for hierarchal (nested) relationships among traits, i.e., indirect, as well as direct, effects of traits on other traits.

The use of path analysis provides the nuance missing from these more standard approaches: directional and hierarchal relationships among traits can be specified and modeled (Shipley 2000). Moreover, one can go further and allow for different relationships among different groups of individuals (multi-group model analysis; Shipley 2000). Another important advantage of the path analytical approach is that one can infer something about ontogeny, specifically that traits are correlated according to certain rules during the growth of the organism.

To provide the most biologically complete picture, a path analysis of phenotypic variation should consider how environmental and genetic variation affect the results. There is a growing (although still small) list of studies that have used a path analytical approach to describe complex patterns of trait variation in *A. thaliana* (Pigliucci and Schlichting 1998; Scheiner *et al.* 2000; Pigliucci and Kolodynska 2006; Scarcelli *et al.* 2007; Tonsor and Scheiner 2007; Wang *et al.* 2009); however, only one of these studies considered genetic variation (Scarcelli *et al.* 2007), and it only investigated two specific single-locus genotypes.

This paper presents a path analytical approach for describing the phenotypic variation among reproductive traits in *A. thaliana*, one that simultaneously accounts for effects of genetic and environmental variation on the relationships among traits. Our study is the most detailed in the botanical path analysis literature that we know of, in terms of the number of various scenarios considered: six different genetic groups, representing the genetic variation of this species along a wide latitudinal gradient in the wild, and four different ecologically significant experimental treatments.

Our definition of reproductive traits refers to those that develop from inflorescence meristems, in contrast to traits that develop from vegetative shoot meristems or root meristems (*sensu* Melzer *et al.* 2008). The transition from vegetative growth to reproductive growth marks an ontogenetic shift in the life cycle of annual plants, a sharp morphological transition from a two-dimensional phenotype (the rosette), which accumulates resources for current survival, to a three-dimensional phenotype (the inflorescences, or flowering stalks, and associated branches), which shunts resources into the production of and provisioning for offspring (Gadgil and Bossert 1970; Cohen 1976; but see Earley *et al.* 2009 for an alternate explanation of their function). Thus the traits produced after this shift are developmentally and functionally distinct from those produced before. We focus in this study on the relationship between architectural traits (i.e., inflorescences originating from the rosette and branches off the inflorescences) and traits more closely related to fitness that are built on these scaffoldings (i.e., fruits, and the seeds within the fruits, that develop on the inflorescences and on the inflorescence branches; referred to here as fitness-related traits).

The plant material used in this study was from a set of populations collected over a broad geographical range in Europe, for which molecular data (unpublished data) was used to group maternal seed families into genetically distinct units. Uniquely, our study parses out the genetic differentiation contributing to path analytical variation. The variation among traits was also modeled separately for plants that experienced different ecologically relevant experimental treatments: low or high nutrients, and apical meristem damage or no apical meristem damage. The nutrient levels were based on the range experienced by *A. thaliana* in nature (H. Callahan, unpublished data, and D. Byers, unpublished data). Apical meristem damage (**Fig. 1**) was used because it is potentially common in the wild: Weinig *et al.* (2003) found that a third of *A. thaliana* plants were apically damaged by rabbits in their field experiment; in other annuals, the natural prevalence of apical meristem damage is even better documented (Paige 1999; Juenger *et al.* 2000). Previous work has demonstrated that nutrient levels variation and apical meristem damage variation affect phenotypic variation in *A. thaliana* (Pigliucci and Schlichting 1998; Weinig *et al.* 2003; Banta *et al.* 2010), but how this environmental variation affects the relationships among traits, and the degree to which the effect of environmental variation on the relationships among traits depends on genetic variation, have not yet been investigated.

Fig. 1 Apical meristem damage to *Arabidopsis thaliana* in the wild (Stony Brook, NY, USA).

This study addresses the following questions: (1) What is the ontogeny of reproductive traits in *A. thaliana*, as inferred from the hierarchal, directional relationships among traits? Specifically, (a) What is the relationship between architectural traits and fitness-related traits? (b) What are the relationships among fitness-related traits themselves? (2) Does ontogeny differ for plants experiencing different nutrient levels and apical meristem damage levels? (3) Does allowing for different trait relationships in different genetically distinct groups change the picture of reproductive ontogeny, and change how it is affected by environmental variation (in the quantitative genetic sense of the term), and if so, what sorts of differences are revealed?

MATERIALS AND METHODS

Plant material and handling, experimental protocol

Maternal seed families (accessions) of *Arabidopsis thaliana* were collected from populations in three different regions (Spain, abbreviated as "SP;" the Netherlands, "NL;" Sweden, "SW"). Many of these accessions are now available from the *Arabidopsis* Seed Stock Center (www.arabidopsis.org). The accessions we used (and their stock center numbers, if available) were: NL3.3 (stock number CS75841), NL3.4, NL3.8 (CS76073), NL5.6 (CS75849), NL5.7 (CS76074), SP1.6, SP1.8 (CS76007), SP1.13 (CS76008), SP5.6 (CS75808), SP5.7 (CS75809), SP6.1 (CS75813), SP6.7 (CS75818), SP8.1 (CS75822), SP8.7 (CS75825), SP8.8 (CS75826), SW1.1 (CS75860), SW1.5, SW2.4, SW2.7 (CS75862), SW7.1, SW7.2, and SW7.3. The two-letter character string and the first number identify the population of origin of the accession, and the

next number identifies the maternal seed family of origin within the population. The populations were sufficiently far from roadsides, railroads, and footpaths to be reasonably considered natural.

We followed the guidelines for germination and growth of *A. thaliana* recommended by the *Arabidopsis* Biological Resource Center (2008). We germinated seeds under laboratory conditions and then used seeds produced by plants raised under controlled conditions to minimize maternal effects. We imbibed the seeds with water on Whatman™ Grade No. 2 moist filter paper in 16 × 50 mm BD Falcon™ tissue culture dishes and exposed them to a seven-day dark stratification treatment at 4°C to facilitate germination. We then planted them in 3.25 × 3.25 × 5 cm pots on two high-intensity light racks (approximately 250 $\mu E/m^2/s^1$ photon flux). While the light intensities were reasonably uniform, the experimental design was fully randomized to prevent any spatial heterogeneity from having a confounding effect. We also rotated the three shelves within each rack weekly to further homogenize the light conditions. In all analyses, we kept track of the light rack on which the plants were grown to account for any systemic differences in the lighting conditions.

Rather than potting soil, we used a 50: 50 mixture of river sand and vermiculite to ensure low baseline soil nutrient levels. When the seeds in a pot failed to germinate, we transplanted a seedling of the same maternal seed family into that pot. The seedling came either from another pot with extra germination or from extra seeds left over from planting, which had germinated in the tissue culture plates and had been kept moist at room temperature since then. In all analysis, we kept track of transplant status to account for the possibility that traits/trait relationships differed for plants that experienced different germination and early growth conditions.

We set the photoperiodic regime to that typical of the Netherlands, roughly in the middle of the geographic range from which these accessions were collected. During seed germination, we set the photoperiod to September 15th (12 h and 44 min), approximately when winter annual ecotypes of *A. thaliana* would be expected to germinate in the field, and used the ambient temperature under the high intensity lights during daytime (~25°C) and nighttime (~20°C). Twenty-five days later, we placed the plants into a walk-in refrigerator for rosette vernalization, with a day-time/nighttime temperature of 4°C and a photoperiod of January 4th-Netherlands (seven hours and 47 min). After six weeks, we returned them to room temperature during daytime and nighttime and set the photoperiod to April 30th-Netherlands (14 h and 52 min). These conditions are a compromise between the need for as natural a setting as possible and the inevitable logistical limitations of experimental designs.

We added nutrients in the form of Scott's™ Osmocote Classic 14-14-14 time-release nitrogen-phosphorus-potassium prills applied to the sand-vermiculite surface. All plants received one prill 11 days after planting. The high-nutrient plants received another seven prills 23 days after planting, about the time the first true leaves appeared. There was some variation in prill size, which probably contributed somewhat to the residual variance in the analyses. Due to the desiccation caused by the fluorescent lights and the poor water-retention ability of the sand-vermiculite mixture, we sub-irrigated the plants twice daily. Therefore, the prills may have leached their nutrients faster than the three-to-four month time interval indicated by the manufacturer. However, an assay of the nutrient levels in the sand-vermiculite mixture, performed approximately three months into the experiment, suggests that this was not a problem. We measured the nitrogen: phosphorus: potassium (NPK) levels, using a LaMotte soil test kit (www.lamotte.com), of ten randomly collected samples of sand-vermiculite from the high nutrient levels treatment and ten random samples from the low nutrient levels treatment. The nutrient levels were still quite high in the high-nutrient treatment, and were sufficient for growth in the low-nutrient treatment (average high nutrients 50: 46: 180 ppm NPK; average low nutrients 7: 18: 129 ppm NPK).

Apical meristem damage (AMD) was carried out at the time of bolting by clipping off the main inflorescence (also known as the apical inflorescence) during the unopened flower bud stage (**Fig. 2**). The entire inflorescence was clipped off at the base of the

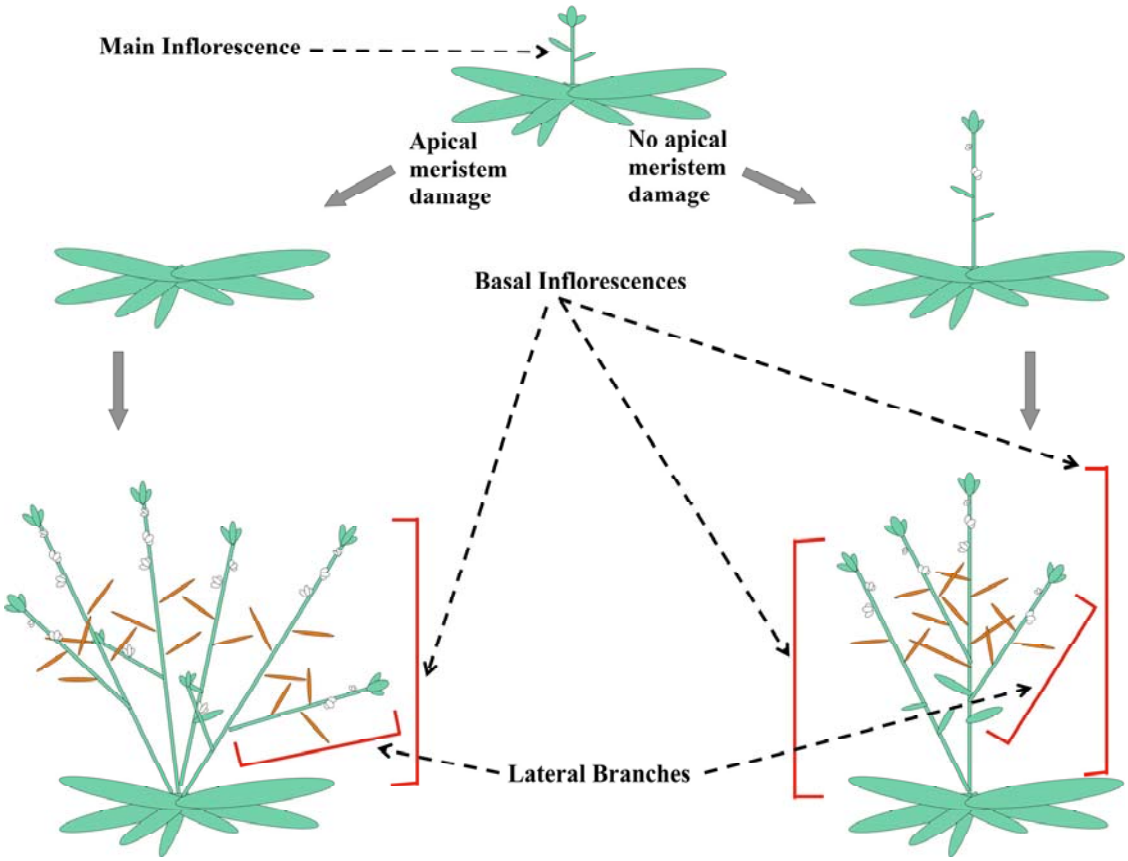

Fig. 2 A diagram showing when during ontogeny apical meristem damage was performed, as well as hypothetical ontogenetic outcomes. Our terminology is depicted as well. The "main inflorescence" is the apical inflorescence formed during the initiation of the reproductive phase of the life cycle. "Basal inflorescences" are any reproductive structures emanating from separate shoot meristems at the base of the rosette; the main inflorescence is considered to be one of the basal inflorescences. "Lateral branches" are branches emanating from a basal inflorescence or from other lateral branches.

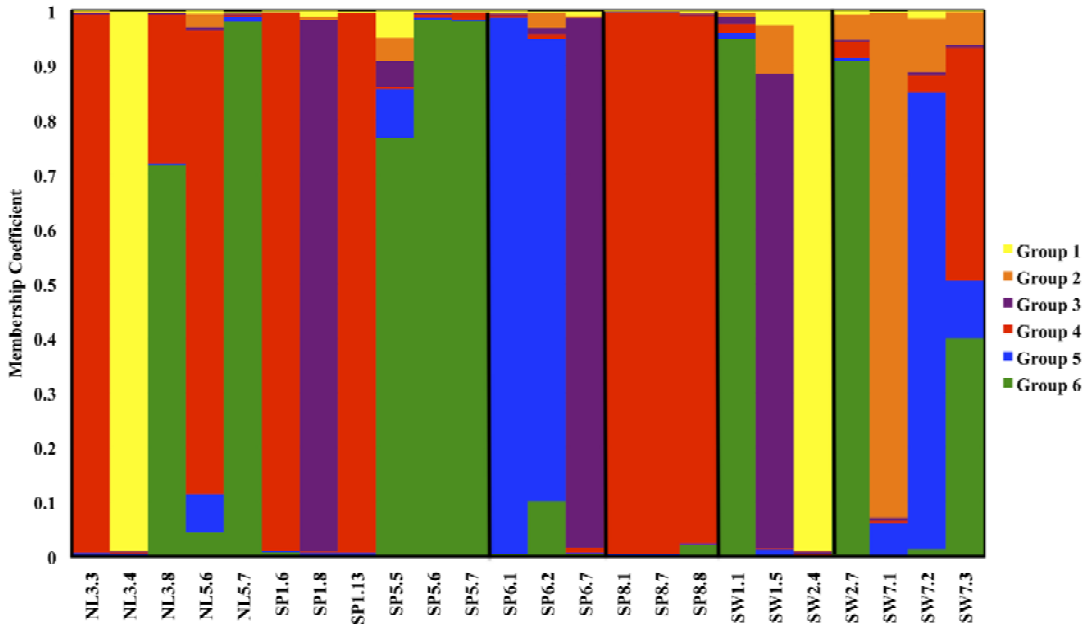

Fig. 3 Membership coefficients for the accessions used in the STRUCTURE analysis. Some accessions listed here (NL3.8, NL5.6, SP5.5, SP6.2, SW1.5, SW7.2, and SW7.3) were not used in the path analyses because they appeared to contain too much admixture from multiple groups.

rosette with scissors while we were careful not to remove or damage any rosette leaves. All plants that survived germination and/or transplanting bolted.

We followed the inflorescence architecture terminology previously used by Banta and Pigliucci (2005), Ehrenreich *et al.* (2007), and Banta *et al.* (2010). After the reproductive period, we measured: two architectural traits related to reproduction: (a) the number of basal inflorescences (lateral branches plus the main inflorescence, if extant) and (b) the number of lateral branches (the branches off of the basal inflorescences and off of other lateral branches themselves) (**Fig. 2**); and three traits more directly related to reproductive fitness: (c) the total number of fruits, (d) the average number of seeds per fruit (determined from a sample of 5 fruits per plant), and (e) seed germinability (determined from a sample of 20-40 seeds tested for germinability per plant). We also measured total plant weight (the sum of the shoot, root, and rosette dry-weights for each plant).

We chose to use inflorescence architecture terminology that does not differentiate between the main inflorescence and other inflorescences emanating from separate shoot meristems in the base of the rosette. According to this terminology, all of these inflorescences are basal inflorescences. We think this is appropriate because half of our plants had no main inflorescence at senescence (due to its experimental removal). Differentiating between the two sub-types of inflorescences would have meant that a trait (the main inflorescence) was only present in half of the plants (the plants that lacked apical meristem damage) and that the trait was invariant (since there is only one main inflorescence); the trait would thus have been uninformative, as it would not have been amenable to any sort of statistical analysis. Another reason for this choice of terminology is that we were interested in the relationships of the architectural traits to fitness-related traits. Relating the remaining (non-main) inflorescences to the fitness-related traits would have been problematic, because the fitness-related traits on the main inflorescence would not have been included in the analysis; this missing fitness could have lead to erroneous or misleading conclusions about whole-organism fitness and about trade-offs among fitness-related traits.

Population genetic analysis

We were interested in genetic effects on the path models we constructed. Because *A. thaliana* is highly selfing and displays large amounts of population structure (King *et al.* 1993; Hardtke *et al.* 1996; Vander Zwan *et al.* 2000), it is not necessarily correct to assume that each inbred individual collected from the wild is truly genetically distinct. Thus it may not be prudent to model every accession separately, since this may result in redundant information and consequently reduce power unnecessarily. It might make more sense to treat genetically similar individuals as the same type and pool them together. Because of these considerations, we grouped our accessions using a Bayesian genetic clustering algorithm, based on overall molecular genetic similarities, and used these groups in the path analytical models, rather than the individual accessions themselves, to test for genetic differentiation in path structure.

We used the program STRUCTURE (Pritchard *et al.* 2000) to determine the number of genetically distinct groups constituting our study. We ran STRUCTURE based on amplified restriction fragment polymorphisms (AFLPs) at 42 informative loci that were scored for each accession (Cruzan *et al.*, unpublished data). We tried different numbers of groups, ranging from 2-20, and found that 6 groups had the highest likelihood (in the statistical sense, i.e., the lowest log-likelihood; Sokal and Rohlf 1995) (data not shown).

Using these results (**Fig. 3**), we assigned accessions to discrete groups. STRUCTURE uses the number of hypothetical groups to assign "membership coefficients" to each accession. The coefficients correspond to the proportion of the genome contributed to that accession by each hypothetical group, and they sum to one. We only considered an accession to belong to a particular group if the membership coefficient was greater than 90%. Based on this criterion, seven accessions (227 plants) were excluded (NL3.8, NL5.6, SP5.5, SP6.2, SW1.5, SW7.2, SW7.3), leaving 17 accessions (398 plants) for further inclusion in our study (NL3.3, NL3.4, NL5.7, SP1.6, SP1.8, SP1.13, SP5.6, SP5.7, SP6.1, SP6.7, SP8.1, SP8.7, SP8.8, SW1.1, SW2.4, SW2.7, SW7.1; **Fig. 3**).

Analyses of variance

To establish that there was significant variation among genetically-distinct groups and treatments that can then be modeled by path analysis, we first performed multi-way analyses of variance (ANOVAs) on each reproductive trait (number of basal inflorescences, number of lateral stems, number of fruits, average number of seeds per fruit, and seed germinability) as a function of: genetic group (based on a membership coefficient > 0.90 from the STRUCTURE analysis; five levels: group 1, group 2, group 3, group 4, group 6), nutrient levels treatment (two levels: low or high), AMD treatment (two levels: damaged or undamaged), and two cofactors, light rack (two levels: one light rack or the other), and transplant status (three levels: non-transplants, pot-to-pot transplants, and Petri dish-to-pot transplants). All effects were

fixed. We included the two cofactors because we found them to be significant sources of variation in another study (Banta *et al.* 2010). To determine which of the experimental treatments (low nutrients, high nutrients, AMD, no AMD), if any, were perceived by *A. thaliana* as stressful, we also performed ANOVA on total plant weight, using the same factors as in the other models.

We checked the data for adherence to the assumptions of normality, homoscedasticity and kurtosis (Sokal and Rohlf 1995), and found that the following transformations were necessary: $\log_{10}$ transformation of number of basal inflorescences, number of lateral branches, and total weight, square root transformation of fruit production, and arcsine-square root transformation of seed germinability.

Path analysis

We created a path analytical model (Shipley 2000) incorporating the reproductive traits used in our study (number of basal inflorescences, number of lateral branches, number of fruits, average number of seeds per fruit, and seed germinability). To remove extraneous sources of variation, we first performed separate ANOVAs on each trait as a function of light rack and transplant status. We then standardized the residuals to a mean of zero and a standard deviation of one, and used these corrected and standardized trait values for path analysis.

Using AMOS version 7.0 (Arbuckle 2003), we employed a phenotypic path model assuming the following causal sequence: basal inflorescence development precedes development of lateral branches, which in turn precedes development of fruits. After the plants initiate fruit development, they commit to making a certain number of seeds and then provision them with resources (where germinability is a proxy for maternal provisioning).

We did not include total plant weight in the path models, because it is a mixture of both architectural traits and fitness-related traits; it is comprised of both the weights of inflorescences and branches and the weights of fruits and seeds. Thus, we believe that including total plant weight in the model would have confused the interpretation of the results. Furthermore, the weights of the plant structures are presumably at least somewhat co-linear with the traits already in the models.

We performed four separate multi-group path analyses (Shipley 2000) for plants experiencing each of four different experimental treatments: (a) low nutrients, (b) high nutrients, (c) AMD, or (d) no AMD. Despite the cross-factoriality of our experimental design, we did not perform multi-group analyses for every possible two-treatment combination, due to insufficient statistical power.

For each multi-group path analysis, the "multi-groups" were the genetically-distinct groups that we defined based on the STRUCTURE analysis. In developing the best-fit models, we specified the genetic group of each plant. Each path in the fully constrained model (one assuming equal regression weights across different genetic groups) was sequentially relaxed to allow for heterogeneity in the path coefficients among genetic groups. We then assessed whether this resulted in an improvement in model fit (measured as a statistically significant decrease in χ^2). Paths were retained as unconstrained when this resulted in significantly improved model fit.

Due to the small sample size of genetic group 5, it was excluded from all analyses. The sample sizes of the genetic groups used in the analyses were as follows: low nutrients, group 1 = 13, group 2 = 21, group 3 = 22, group 4 = 83, group 6 = 55; high nutrients, group 1 = 16, group 2 = 24, group 3 = 18, group 4 = 83, group 6 = 47; damaged, group 1 = 15, group 2 = 23, group 3 = 19, group 4 = 79, group 6 = 50; undamaged, group 1 = 14, group 2 = 22, group 3 = 21, group 4 = 90, group 6 = 52.

The genetically-distinct groups we defined harbor different amounts of molecular genetic diversity, due to different numbers of accessions comprising the distinct groups. This could translate into different distributions of trait values in distinct genetic groups. One concern, therefore, might be that the tests for genetic differentiation in the path coefficients are not valid because of heteroscedasticity in the data. While heteroscedasticity is an issue in ANOVA-type approaches, multi-group path analysis makes no assumptions about homoscedasticity (or lack thereof) when testing for differences among groups (Shipley 2000). Another related concern might be that heteroscedasticity affects the estimation of the path coefficients themselves. A visual inspection of the trait distributions revealed that they were homoscedastic, both within individual genetic groups and across genetic groups (data not shown).

For the multi-group models, we calculated several metrics of model performance, specifically: the P-value of the χ^2 statistic (Shipley 2000), the root mean square error of approximation (Arbuckle 2003), and the comparative fit index (Bollen 1989).

RESULTS

STRUCTURE analysis

The STRUCTURE analysis revealed discordant patterns of genetic similarity and geography (**Fig 2**). While two populations (in the geographic sense) contained accessions with similar membership coefficients (populations SP5 and SP8; **Fig. 3**), the other populations contained accessions with very different membership coefficients (e.g., NL3 and SW2; **Fig. 3**). Furthermore, accessions with very similar membership coefficients were often located in disparate populations (e.g., accessions SW2.4 and NL3.4; **Fig. 3**).

Analyses of variance

All ANOVA models were highly significant ($P < 0.0001$, not shown). Effects of genetic group were significant for all traits, effects of nutrients were significant for all traits except seed germinability, and effects of apical meristem damage (AMD) were significant for all traits except lateral

Table 1 Analyses of variance for six traits. For each effect (first column), we present sums of squares and F-ratios (columns) and degrees of freedom (parentheses).

Effect	*SS*	*F*	*SS*	*F*	*SS*	*F*
	Basal Inflorescence No.		***Lateral Branch No.***		***Fruit Production***	
K-group (5)	0.87	2.52*	5.07	4.17*	655.26	17.18***
Nutrient Levels (1)	6.15	89.42***	37.07	152.43***	1129.42	148.03***
Apical Meristem Damage (1)	1.81	26.29***	0.020	0.082	36.29	4.76*
Light Rack (1)	0.17	2.54	0.60	2.47	273.97	35.91***
Transplant Status (2)	0.0057	0.041	0.041	0.084	56.94	3.73*
Error	26.91 (391)		95.09 (391)		2952.70 (387)	
	Seeds Per Fruit		***Seed Germinability***		***Total Weight***	
K-group (5)	11615.78	28.83***	7.10	8.59***	0.15	7.77***
Nutrient Levels (1)	3543.34	43.97***	0.032	0.19	0.38	94.68***
Apical Meristem Damage (1)	2425.40	30.10***	0.18	1.07	0.080	20.03***
Light Rack (1)	2980.62	36.99***	0.80	4.82*	0.032	8.16*
Transplant Status (2)	410.45	2.55	0.10	0.29	0.006	0.78
Error	31026.72 (385)		62.82 (380)		1.54 (387)	

*P < 0.05
**P < 0.001
***P < 0.0001

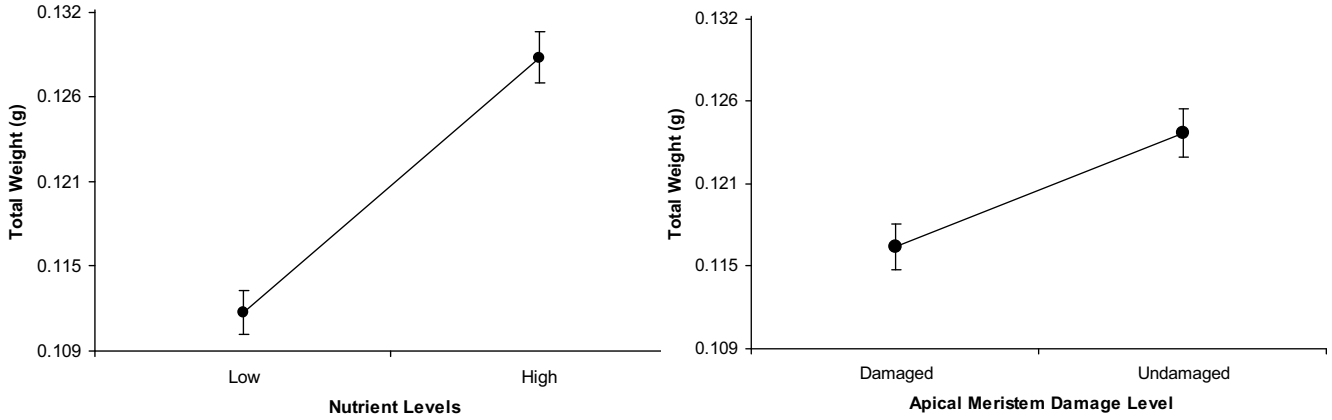

Fig. 4 Least squares mean total weights of plants at different nutrient and apical meristem damage levels. The error bars are ±1 SE.

branch number and seed germinability (**Table 1**). Taken together, these results indicate that there was significant phenotypic variation among plants from genetically-distinct groups and among different experimental treatments that can be modeled using path analysis.

The cofactors light rack and transplant status, which were included to account for extraneous sources of variation in the analysis, were sometimes significant. Specifically, light rack effects were significant for four traits (fruit production, seeds per fruit, seed germinability, and total weight) and a transplant effect was significant for one trait (fruit production), although the effect size (measured in sums of squares) was small relative to the other significant sources of variation and relative to the residual variance.

The ANOVA of total weight revealed that both nutrient levels and AMD affected the size of the plants, with low nutrient levels and AMD resulting in smaller plants than high nutrient levels and no AMD (**Fig. 4**). Assuming that more stressful treatments result in smaller plants, this indicates that the plants perceived low nutrient levels and AMD to be more stressful than high nutrients and no AMD, as expected.

Path analytical models

χ^2 statistics for the best-fit models were significant ($P < 0.0001$ for all models; data not shown), indicating that the models did not account for enough of the variance-covariance in the data to be considered good fits (Shipley 2000). The other metrics of model fit were also not ideal (root mean square error of approximation values ranging from 097 – 0.22, comparative fit indices ranging from 0 – 0.53; data not shown). We believe the lack of good fit is probably due, at least in part, to the fact that we did not model every possible combination of treatment levels separately. In each model, the variation attributable to an axis of environmental variation (either nutrient levels variation or the variation in AMD) was not accounted for, decreasing the fit of the data to the particular model. In spite of this problem, we still managed to detect many significant path coefficients, as well as genetic differentiation in path coefficients.

Path coefficients in the multi-group path models differed among genetic groups and among experimental treatments (**Fig. 5**). Overall, a few generalizations about the path models can be made. First of all, there are two particular paths that are always significant: the relationship between basal inflorescence number and later branch number, and the relationship between fruit production and the number of seeds per fruit (**Fig. 5**). In both cases, the relationship is positive. Second of all, there are always some positive and some negative downstream effects of the architectural traits on the fitness-related traits, suggesting trade-offs between architecture and fitness (**Fig. 5**).

Another interesting feature of the path models is that there is no evidence of trade-offs, in the form of negative path coefficients, among the fitness-related traits themselves (**Fig. 5**). Seed germinability was not even affected, positively or negatively, by the other fitness-related traits in some experimental treatment-genetic group combinations (**Fig. 5B**, **5D**).

While low nutrients and apical meristem damage seemed to be the most stressful treatments, as indicated by the differences in total weight between these treatments and their less stressful counterparts, the differences in the path models do not seem to be easily understood as merely a stress response syndrome induced by the harsher treatments. While the model for plants at low nutrients and the one for plants with AMD share the same negative path and corresponding path coefficient, and also share a statistically indistinguishable path coefficient for the relationship between lateral branch number and seed germinability for group 2 (**Fig. 5A, 5C**), these models also have differences. Specifically, the model for plants with AMD revealed a significant path between basal inflorescence number and fruit production, and also a very strong positive relationship between fruit production and seed germinability for group 1, but neither of these relationships were replicated in the model for plants at low nutrients (**Fig. 5A, 4C**).

DISCUSSION

The lability of the relationships among traits

We demonstrated that the relationships among architectural traits and fitness-related traits vary depending on the genetic background and experimental treatment. While previous work with *A. thaliana* has shown that traits' path model coefficients can vary among experimental treatments (Pigliucci and Schlichting 1998; Pigliucci and Kolodynska 2006; Tonsor and Scheiner 2007), the lability of these path coefficients to the overall genetic background-experimental treatment combination has not been sufficiently investigated in this experimentally valuable species (but see Scarcelli *et al*. 2007). In fact, this phenomenon has never been studied over a wide range of genotypes combined with more than two experimental treatments.

It was far from obvious *a priori* that the relationships among traits would be labile to the genetic background, given that allometries (stable character-character correlations) are commonly observed in nature (Reiss 1991; Niklas 1994) and that organisms are expected to be developmentally canalized and buffered against genetic mutations and variation in order to maintain a coherent phenotype (Wagner *et al.* 1997; Hall 1998; Schwenk and Wagner 2001; West-Eberhard 2003b; Bagheri and Wagner 2004; Preston and Ackerly 2004).

Our study does not address the precise genetic mechanisms accounting for the differences in the relationships

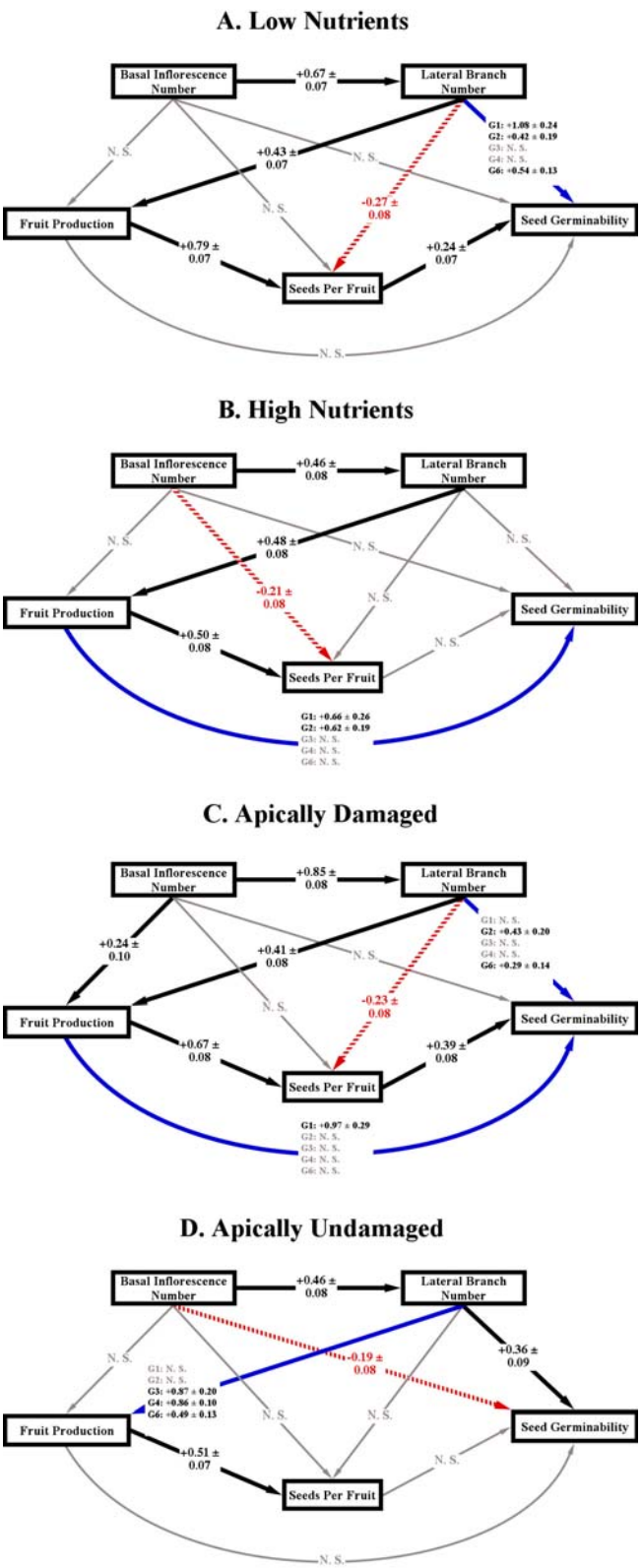

Fig. 5 Multi-group path analysis models for plants experiencing one of four different treatments: (A) low nutrient levels, (B) high nutrient levels, (C) apical meristem damage, or (D) no apical meristem damage. Black lines indicate a path coefficient was significantly positive and red dashed lines indicate it was significantly negative. Blue lines indicate a path coefficient varied among the genetically-distinct groups (represented as G1, G2, G3, G4, and G6), and gray lines indicate a path coefficient was not significant. Path coefficient estimates and standard errors are presented, when significant.

among traits that we observed. There are several candidate genomic regions/genes (i.e., candidate quantitative trait loci, or QTLs) for inflorescence production, lateral branching, and fruit production that have been identified in *A. thaliana* (e.g., Ungerer *et al.* 2002; Ungerer *et al.* 2003; Weinig *et al.* 2003b; Olsen *et al.* 2004; Hanzawa *et al.* 2005; Ehrenreich *et al.* 2007; Scarcelli *et al.* 2007). One could test whether these QTLs affect the relationships among traits (Kelly 2009; see Brock *et al.* (2009) and Scarcelli *et al*. (2007) for empirical examples).

Genetic differentiation

The grouping of our accessions by overall genetic similarity did not match the groupings of our accessions by geographic proximity (i.e., individuals within the same geographic population were not necessarily closely related to one another). This demonstrates that it is not always prudent to use population of origin (i.e., geography) as a proxy for relatedness. In fact, our study shows that it is not even safe to assume that one population has the same relationships among traits; thus restricting the geographic range of the sample being considered will not necessarily homogenize the results.

Are path model differences due to a "stress response" phenotype?

While we found that low nutrient levels and apical meristem damage were, not surprisingly, perceived by the plants as the most stressful experimental treatments (assuming smaller plants are more stressed), this information did not lead to a straightforward interpretation of our path model results. Specifically, the path model differences cannot be explained as the outcome of two qualitatively distinct ontogenetic trajectories, one normal and one activated by stress. This might have been the case if the inflorescence ontogeny were highly canalized, and were only perturbed in specific, predictable ways caused by the elicitation of a generalized stress response. Instead, any sort of stress response underlying our results is surely complex. One possibility is that low nutrients are perceived by the plant as a different kind of stress than apical meristem damage, and therefore different genetic stress-response mechanisms, with different constellations of effects, are elicited in each instance (sensu Scharloo 1991; Bergman and Siegal 2003; Mittler 2006). Fine-scale molecular studies would be needed to determine the transcriptomics and genetic architecture of the relationships among traits found here.

Evolutionary implications

The ontogenetic variation just described has potentially important evolutionary consequences. In microevolutionary terms, it has ramifications for the response to selection on reproductive ontogeny. Within each experimental treatment there appear to be some basic "rules of form" (Hall 1998). For instance, at low nutrients, plants that grow more basal inflorescences tend to grow more lateral branches, which in turn leads to more fruits but also to fewer seeds per fruit, and the number of seeds per fruit positively affects the quality of the individual seeds. If these statistical relationships do, in fact, represent a developmental constraint (in the sense that only certain combinations of trait values are possible; Wallace 2003), then this amounts to a constraint on the power of selection to move *A. thaliana* populations to a different ontogenetic trajectory. But even if these constraints turn out to be real, their significance over evolutionary timescales would require verification with a larger number of samples, and a phylogenetic approach to determine whether the relationships among the traits in question are conserved over a significant amount of time.

Our study also demonstrates that there is differentiation in the relationships among traits for different genetically distinct groups – i.e., genetic differentiation in some path coefficients. Because genetic variation is the fuel that converts selection pressures into evolutionary changes

(Falconer and Mackay 1996; Hartl and Clark 1997), our results suggest that selection for different inflorescence ontogenies can result in microevolutionary changes. For instance, if it were selectively advantageous at low nutrient levels for lateral branch number and seed germinability to be decoupled from one another, *A. thaliana* would potentially be able to evolve in that direction.

There is also a potential macroevolutionary implication of this work. Since we found that the relationships among traits differed to such a large extent among different experimental treatments and among genetically-distinct groups, this suggests that any developmental constraints are actually quite local (although Schwenk and Wagner 2004 point out that all constraints are, in fact, relative, i.e., local, to one degree or another). In other words, it appears possible, and indeed relatively easy, to break these constraints. Furthermore, Earley *et al*. (2009) found that inflorescence architecture is a very important determinant of lifetime net carbon gain in *A. thaliana*. Taken together, this leads to the expectation that species related to *A. thaliana* may have evolved very different inflorescence ontogenies in response to different ecological pressures (as it is actually observed; Pigliucci *et al.* 1999; Banta 2008). West-Eberhard (2003b) and others (e.g., Gibson and Wagner 2000) have advanced the notion that relatively small changes in the genotype and/or the particular circumstances of an organism can result in seemingly much more complex, coordinated changes in the phenotype that are observed over macroevolutionary time scales. The apparent ease with which different reproductive ontogenies originate in *A. thaliana* supports this notion.

The relationships among fitness-related traits

We found, surprisingly, that the fitness-related traits showed no negative relationships among one another. This is interesting because life history theory predicts that components of fitness should be negatively correlated with one another (Houle 1991). One possible explanation for the discrepancy between our results and theoretical expectations is that variation in the size of the plants is much larger than the variation in the fitness-related traits, so that variation in plant size is masking the ability to detect negative covariances among fitness-related traits (Houle 1991). This possibility warrants an entire follow-up study of its own.

It is important to note that the exact relationship among fitness-related traits differed depending on the experimental treatment and on the genetic background, so that our results cannot be taken to show that fruit production is a reliable predictor of the other fitness-related traits. This is decidedly not so, given also that the architectural traits have negative effects on some fitness-related traits but not on others; thus any positive effect of fruit production on the other fitness-related traits is at least partially cancelled out by the negative effects of the architectural traits on those same fitness-related traits.

Reproductive ontogeny and tolerance to apical meristem damage

Many studies have reported the surprising finding that apical meristem damage can actually increase fitness, relative to undamaged controls, or that at least plants can mitigate the fitness loss so that it is less than what might be expected. This phenomenon is known as tolerance to apical meristem damage (Paige and Whitham 1987; Weinig *et al.* 2003; Wise and Abrahamson 2008; Banta *et al.* 2010). The conventional wisdom is that increased tolerance is due to the proliferation of inflorescences caused by the disruption of apical dominance, which results in increased fruit production due to the increased number of basal inflorescences.

While we did not measure tolerance to apical meristem damage in this study (see instead Banta *et al.* 2010), our findings suggest that the causal scenario linking architecture to fitness is much more complicated than this conventional wisdom. The exact degree to which apical meristem damage results in an increase in "true" fitness apparently depends on the genetic background of the plants, and these dynamics are almost completely invisible to a tolerance study that focuses solely on fruit production as the metric of fitness. Furthermore, basal inflorescence production alone does not account for fitness, and thus apical meristem damage cannot necessarily be assumed to be beneficial simply because it increases the number of basal inflorescences.

CONCLUSION

In summary, our results demonstrate that complex characters, such as those relating to reproduction in *Arabidopsis thaliana*, can be realized in multiple ways by different combinations of the underlying constituent traits. This situation is analogous to the repeated finding at the genetic level that traits can be realized in different ways by different combinations of the underlying constituent genes and genetic processes (e.g., Komeda 2004; Wilson *et al.* 2005; Smith and Boyko 2007; Heinzen *et al.* 2008; McCarthy *et al.* 2008), and highlights the fact that networks of interacting phenotypes, like gene networks, can be complex, hierarchal and contingent on the genetic background. Considering the variety of patterns that can be generated by combining genetic and environmental sources of variation, it is not surprising that the goal of fully understanding the dynamics that generate genotype-to-phenotype mapping have been elusive (Pigliucci 2003c; Korbel *et al.* 2005; Hammer *et al.* 2006). We suggest that investigations into the phenotypic aspects of model organisms are just as enlightening and necessary as investigations into molecular complexity, and should therefore represent a priority for research on model organisms.

ACKNOWLEDGEMENTS

We would like to thank Rachael Aletti, Davide Arcuri, Claude Banta, Christia Brinkley, Lori Cooke, Paula Crouse, Warren Denning, Romina Dimarco, Matthew Figliola, Leah Gordon, Rocky Graziose, Deanna Guitierrez, Gareth Mead, Kristine Miranda, James Mynes, Tilhami Qureshi, Ekaterina Shevchenko, Catie Stewart, and Walter Whitworth for their help with the plant care, data collection, and data analysis. We would also like to thank Ken McFarland, greenhouse manager at the University of Tennessee-Knoxville, Michael Axelrod and John Klumpp, greenhouse managers at Stony Brook University, and Michael Doal, manager of the Functional Ecology Research and Training Laboratory (FERTL) at Stony Brook University, for logistical assistance. We are very appreciative of Diane Byers and Hilary Callahan, for providing information about inorganic soil nutrient levels, of Norris Muth, for assisting with structural equation modeling, and of Jeanmarie Molina and Jonathan Flowers, for assisting with the population genetics aspect of the project. Conversations with Doug Futuyma about tolerance to apical meristem damage were very important in shaping this manuscript. Jefferey Dole collected the seeds from Europe that were used in this experiment. This work was supported by NSF Grant DEB-0089493 to MP.

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