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ORIGINAL PAPER

Heritable variation in the inflorescence replacement program of *Arabidopsis thaliana*

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Abstract Owing to their sessile habits and trophic position within global ecosystems, higher plants display a sundry assortment of adaptations to the threat of predation. Unlike animals, nearly all higher plants can replace reproductive structures lost to predators by activating reserved growing points called axillary meristems. As the first step in a program aimed at defining the genetic architecture of the inflorescence replacement program (IRP) of *Arabidopsis thaliana*, we describe the results of a quantitative germplasm survey of developmental responses to loss of the primary reproductive axis. Eighty-five diverse accessions were grown in a replicated common garden and assessed for six life history traits and four IRP traits, including the number and lengths of axillary inflorescences present on the day that the first among them re-flowered after basal clipping of the primary inflorescence. Significant natural variation and high heritabilities were observed for all measured characters. Pairwise correlations among the 10 focal traits revealed a multi-dimensional phenotypic space sculpted by ontogenic and plastic allometries as well as apparent constraints and outliers of genetic interest. Cluster analysis of the IRP traits sorted the 85 accessions into 5 associations, a topology that establishes the boundaries within which the evolving Arabidopsis genome extends and restricts the species' IRP repertoire to that observable worldwide.

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Introduction

The transition from vegetative to reproductive development in many flowering plants is accompanied by a conversion of the shoot apical meristem (SAM) from an exclusively vegetative to a predominantly floral axis-generating unit. Damage to or loss of the primary floral axis (inflorescence) from herbivory, trampling, disease, wind or freezing in a monocarpic, monopodial species, such as *Arabidopsis thaliana* can incur a catastrophic fitness penalty. Whereas chemical and mechanical defenses represent evolved responses to biotic impediments to reproduction, the incompleteness of protection afforded by such adaptations coupled with unavoidable abiotic threats that remain resulted in the evolution of contingent developmental pathways of reproduction. Such accommodation occurs in Arabidopsis and most angiosperms via the activation of dormant axillary meristems (AMs) that develop at the adaxial base (the axil) of vegetative leaves.

In Arabidopsis, the products of AM activation emerge as axillary inflorescences or paraclades (terminology used interchangeably in this report) (Grbic and Bleecker 2000; Leyser 2005; Long and Barton 2000). AMs ubiquitously play the additional role of elaborating plant architecture by

mediating axial branching for maximizing light capture, a role that may evolutionarily predate that of herbivory compensation (Bennett and Leyser 2006). In Arabidopsis, branching of the primary inflorescence axis is mediated by activation of AMs subtended by cauline leaves. The more developed axillary meristems in Arabidopsis, those subtended by basal rosette leaves, do not usually become activated until the primary inflorescence axis displays some degree of elongation. Such delayed activation is a hallmark of apical dominance (Cline 1991; Napoli et al. 1999). Auxin is a primary signal governing the activity of AMs (Thimann and Skoog 1933). The standard model states that when the SAM's integrity is compromised, the primary source of auxin is lost and, relieved of inhibition, axillary buds develop into branches. Phenotypes of Arabidopsis mutants defective in auxin responses [*axr1*, (Lincoln et al. 1990)] and polar transport [*pin1*, (Aida et al. 2002)] support this model (McSteen and Leyser 2005). However, auxin's role in controlling bud activity is indirect (Napoli et al. 1999).

Genetic and physiological analyses in a variety of plant systems have established a regulatory framework for apical dominance and AM activity control (Doebley et al. 1997; Doust 2007; Doust et al. 2004; Komatsu et al. 2003; Kurata et al. 2005; Otsuga et al. 2001; Reintanz et al. 2001; Schmitz et al. 2002; Schumacher et al. 1999; Shimizu-Sato and Mori 2001; Stafstrom 1993). A phylogenetically conserved model describes the network regulating transitions between states of AM activity in dicotyledonous plants (Angenent et al. 2005; Beveridge 2006; Beveridge et al. 1996, 1997, 2003; Foo et al. 2005; Johnson et al. 2006; Leyser 2005; Morris et al. 2001; Ongaro and Leyser 2008; Rameau et al. 2002; Shimizu-Sato and Mori 2001). In Arabidopsis, the *MAX3* and *MAX4* (more axillary branching) genes encode enzymes that cleave carotenoid substrates and *MAX1* encodes a cytochrome P450 oxygenase that acts on a downstream product of MAX3 and MAX4 activities (Auldridge et al. 2006; Booker et al. 2004, 2005; Ehrenreich et al. 2007). The physiologically active product of the MAX3/4/1 biosynthetic pathway (MDS, MAX-dependent signal) is a chemical messenger of carotenoid origin the synthesis or transport of which may be regulated by auxin (Bennett et al. 2006; Simons et al. 2007). Recent studies have suggested that the branch-inhibiting mobile signal is a strigolactone (Umehara et al. 2008).

The mechanism by which the mobile signal retards bud outgrowth involves local proteolytic activity and long-range auxin transport. *MAX2* (and *RMS4*) acts locally in the aerial portion of the plant and encodes a leucine-rich repeat F-box component of an SCF complex that functions in ubiquitin-targeted proteolysis (Ehrenreich et al. 2007; Stirnberg et al. 2002, 2007; Woo et al. 2001). All *max* mutants show enhanced expression of *PIN* auxin efflux carriers and thus elevated polar auxin flow (Bennett et al. 2006). The current model depicts a lateral bud's growth status primarily contingent upon the strength of the adjacent stem vasculature as an auxin sink. Increased capacity for polar auxin transport (e.g., in *pin* or *max* mutants) or depletion of the system's auxin load by removal of the source (decapitation) enables greater efflux from axillary sources, freeing buds to break (Bennett et al. 2006).

Whereas mutant analysis provides essential elements of genetic and physiological regulatory frameworks, the study of species-wide natural variation in a complex trait such as AM behavior can provide evolutionary insights and further genetic details (Koornneef et al. 2004). In a species with as geographically broad an adaptive range as that of Arabidopsis, the spectrum of extant, continuous phenotypic variation for many traits of interest can be exploited with quantitative genetic and genomic tools (Aranzana et al. 2005; Borevitz and Nordborg 2003; Tonsor et al. 2005; Weigel and Nordborg 2005; Zhao et al. 2007). The principal objective of this study was to test the null hypothesis that no significant natural genetic variation exists for the inflorescence replacement program (IRP) in Arabidopsis. We have therefore examined natural variation in the IRP that follows removal of an Arabidopsis plant's primary inflorescence. We report here substantial, heritable variation for elements of the Arabidopsis IRP among a collection of accessions chosen to facilitate subsequent genetic analysis of this complex and evolutionarily vital suite of characters.

Materials and methods

Plant material

Eighty-five accessions of *Arabidopsis thaliana* (see Supplementary Table I) were selected for this study based on several criteria. First, accessions were selected to represent a wide geographic distribution with respect to source populations. Second, accessions chosen were biased toward, but not exclusively confined to, rapid cycling summer annuals in order for their phenotypic characterization to be completed within a reasonable period of time and within the confined space of our planting trays without excessive stress to them. Third, several accessions were chosen based on annotations in the online catalog of the Arabidopsis Biological Resource Center (ABRC, Columbus, OH, USA) indicating a novel architectural feature or milieu of origin. Fourth, in order to facilitate subsequent genetic analyses, accessions were chosen from among the 96 lines that have been the focus of the NSF 2010 project, "A Genomic Survey of Polymorphism and Linkage Disequilibrium" (Aranzana et al. 2005; Nordborg et al. 2002; Zhao et al. 2007). Some accessions among these 96

flowered too slowly despite vernalization and were therefore excluded as impractical for use in this study. Seed stocks of all accessions were obtained from the ABRC and were increased by single seed descent from initially screened samples.

All seeds were imbibed and stratified for 4 to 7 days at 4°C in the dark to break dormancy. Imbibed seeds were sown directly in 4 × 9 cell Compak™ trays (style 36-01) prepared with a 3:1 (vol:vol) mixture of Sunshine LC1™:Vermiculite (approximately 100 cm^3 of soil/plant), pre-wet with a 10 ml/gal Gnatrol™/water solution to control fungus gnat (*Bradysia coprophila*) infestation and thinned to one plant per cell. Trays were positioned on four identical lighted racks in a growth room. Each 48″ w × 24″ d x 84″ h rack consisted of five shelves, each with four, evenly spaced 48″ T8 cool white, high output fluorescent lamps (32 W, 4100°K) positioned 30-cm above the soil surface of the pots on the shelf below. Growing conditions were 18–20°C (day and night), with 16-h days (100–120 μE/m^2/s)/8-h nights with building HVAC humidity control. Trays were watered from beneath and fertilized at 7 and 14 days after germination with Peters™ 15–30–15 (N–P–K) fertilizer.

Experimental design

The germplasm survey was carried out in multiple environments. These environments differed only in the personnel taking data, not in growth conditions. Owing to the scale of the study, the survey had to be carried out serially, in three consecutive segments. Plants were grown in two to three environments during each of these segments. Each environment, in this study, consisted of a single rack of five lighted shelves consisting of three replicate shelves of plants to be clipped (treatment) and two replicate shelves of plants that remained unclipped (control). Each replicate shelf consisted of four incomplete blocks (one Compak™ 36-01 tray per block) with each block (tray) organized into nine plots (accessions) with four plants per plot. Each replicate shelf of 30 accessions was randomized using an alpha lattice design. Each environment could thus accommodate 90 accessions clipped (three shelves of 30 accessions/shelf) and 60 accessions unclipped (two shelves). This design permitted the evaluation of 40–60 plants per accession. The accessions Ler-0 and Col-0 were included in triplicate in each replicate shelf as a check for variability and consistency of measurements. Clipping was performed by severing the primary inflorescence at its base with a pair of fine scissors on the first day that it bore a fully opened flower or reached a height of 4 cm from its base, whichever came first. For each plant, data were recorded for parameters shown in Table 1. All names of traits measured in unclipped plants end in "1", those in clipped plants, "2".

Traits measured were categorized as life history (LH) or response (IRP) traits. LH traits were assessed for 83 accessions, whereas 85 genotypes were evaluated for IRP traits (Supplementary data Table I). Flowering date was that on which a nascent floral axis was discernable at the center of the rosette by the naked eye. Leaf counts were made at flowering and were confirmed by dissection at the end of the experiment. Axillary inflorescences were scored for traits ACL (number of axillaries on day of clipping of primary inflorescence) and ARF (number of axillaries on day of virtual re-flowering) only if they exceeded 0.3 cm in length. It should also be noted that the primary inflorescence was included in the scoring of all LH traits.

The re-flowering date of clipped plants was defined as the day that the first fully opened flower appeared on an axillary inflorescence after the primary inflorescence had been removed. Unclipped plants cannot "re-flower". Therefore, a time point needed to be chosen for taking measurements on unclipped control plants at "re-flowering". This time point was determined by pairing each unclipped control plant with a clipped plant of the same

Table 1 Classes, abbreviations, units and descriptions of traits measured in this study

Class	Abbr.	Unit	Description
Life history (LH)	DFL	Days	Time interval between sowing and appearance of inflorescence at center of rosette
	LFN	#	Number of rosette leaves on the day of clipping
	ACL	#	Number of axillary inflorescences ≥0.3 cm on day of clipping of primary inflorescence
	ARF1	#	Number of axillary inflorescences of ≥0.3 cm on day of virtual re-flowering[a]
	APL1	cm	Length of longest inflorescence on day of virtual re-flowering[a]
	AXL1	cm	Average length of all axillary inflorescences ≥0.3 cm on day of virtual re-flowering[a]
Response (IRP)	ARF2	#	Number of axillary inflorescences of ≥0.3 cm on day of re-flowering
	APL2	cm	Length of longest inflorescence on day of re-flowering
	AXL2	cm	Average length of all axillary inflorescences ≥0.3 cm on day of re-flowering
	DRF	Days	Time interval between clipping and re-flowering

[a] See text for description of "virtual" re-flowering

accession and counting how many days after clipping the clipped plant re-flowered. Its unclipped partner was then measured on a day the interval of which since flowering equaled that between flowering and re-flowering in the clipped partner plant. To differentiate this time point from actual re-flowering in clipped plants, we refer to it as "virtual" re-flowering.

Statistical analysis

Data were recorded in notebooks as phenotypes were scored and transferred to Excel™ spreadsheets for preliminary inspection and consolidation. Datasets from all germplasm evaluations were combined and best linear unbiased predictors (BLUPs) were determined for each of the 10 LH and IRP traits for all accessions. An analysis of variance was performed applying the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_{ji} + \gamma_{kji} + \delta_l + \varepsilon_{ijkl}$$

where Y_{ijkl} represents the phenotypic mean of a genotype, α_i is the effect of the ith environment, β_{ji} is the effect of the jth replication in the ith environment, γ_{kji} is the kth block effect in the jth replication of the ith environment, δ_l is the effect of the lth genotype, and ε_{ijk} represents the residual error. All effects in the model were considered random. Estimates of the genotypic variance (σ^2_g), phenotypic variance (σ^2_p) and error variance (σ^2_ε) and their standard errors were calculated as described by Searle (1971). Broad sense heritability estimates (h^2) for the RILs were calculated on an entry-mean basis as described (Hallauer and Miranda 1981). Phenotypic correlation coefficients were calculated among traits by applying standard methods (Mode and Robinson 1959). PLABSTAT (Utz 1998) and SAS 9.1 (SASInstitute 1998) software packages were used for all statistical analyses.

A k-mean cluster analysis was performed for a varying number of maximum clusters ($0 \leq k \leq 30$). Cluster cubic criterion (CCC) and the pseudo-F (psF) statistics were calculated for each k and plotted against k. We selected the smallest k with a local maximum for both statistics as the number of clusters present in our set of genotypes. For $0 \leq k \leq 30$, CCC and psF curves indicated five clusters with a local maximum at $k = 5$ for IRP traits.

A principal component analysis (PCA) was conducted on all IRP traits within each of the five IRP clusters to identify cluster-specific patterns. These patterns were defined by the sign and the magnitude of the loadings of the first three principle components. Before performing PCA, the trait values were standardized to have a mean zero and variance one to avoid any scaling effects. PCA was performed on covariance matrices rather than correlation matrices to minimize the influence of any irregularities and measurement error that can arise with standardized variables.

Results

Summary statistics and trait distributions

Inflorescence replacement is a developmental pathway driven by a network of physiological, cellular and biochemical components, all ultimately under genetic control. In order to maximize broad sense heritabilities and thereby enhance resolution of genetic components of variance, a common garden approach was taken wherein 85 Arabidopsis accessions were grown in a replicated design in a controlled environment growth room. Plant responses to inflorescence removal were parsed into discrete component characters. Four traits were measured that explicitly represent inflorescence replacement (IRP). In addition, six life history (LH) traits were also measured in each plant (Table 1). Three LH traits were measured on both clipped and unclipped plants: days to flower, DFL; rosette leaf number, LFN; and number of axillary inflorescences on the day of clipping, ACL. Three IRP traits were measured on both clipped and unclipped plants: number of axillary inflorescences on day of re-flowering, ARF (ARF1 and ARF2, clipped and unclipped plants, respectively); mean length of all axillary inflorescences on day of re-flowering, AXL (AXL1 and AXL2); and, length of the longest axillary inflorescence on day of re-flowering, APL (APL1 and APL2). On unclipped plants, ARF1, AXL1 and APL1 were measured on the day of virtual re-flowering (see "Materials and methods" for a definition of "virtual" flowering). One IRP trait was scored only on clipped plants: days to re-flower, DRF.

Substantial intraspecific phenotypic variation was detected for all characters (Table 2), with trait minima and maxima separated by an average of 5.2 standard deviations (range 4.3–7.4) for the 10 traits analyzed. Broad sense heritabilities were very high for all traits, ranging from 0.66 to 0.98. These results suggest a significant interaction between accessions and treatments, both clipped and unclipped, and that uniform growth conditions minimized differential environmental impacts on replicates.

The range of recorded flowering times (DFL) confirms that our study sampled a broad distribution of early- to late-flowering accessions (Clarke et al. 1995; Karlsson et al. 1993; Kowalski et al. 1994; Kuittinen et al. 1997). DFL varied from 28.6 days (Ren-1) to 103.8 days (Rmx-A02) across accessions grown under our long-day conditions, with a mean of 51.8 days. Rosette leaf number and flowering time are among the quintessential life history covariates in studies of Arabidopsis evolutionary ecology (Camara et al. 2000; Lempe et al. 2005; Mitchell-Olds 1996; Pigliucci 2003; Pouteau et al. 2004) and indeed the LFN–DFL correlation was highly significant ($r = 0.61$**;

Table 2 Mean, ranges, estimates of variance components and heritabilities for life history and IRP traits

Life history						
Trait[a]	DFL	LFN	ACL	ARF1	APL1	AXL1
Unit	Days	#	#	#	cm	cm
Mean	51.8	22.5	0.09	2.0	25.1	3.8
Min–Max	28.6–103.8	9.7–41.5	0.01–0.86	0.6–4.8	12.8–34.9	1.4–7.0
Std Dev	17.5	7.3	0.14	1.0	3.8	1.72
Variance components						
$\hat{\sigma}^2_g$	300.75	53.72	0.03	1.53	9.61	3.45
$\hat{\sigma}^2_{ge}$	1.84	3.99	0.02	0.00	0.47	0.15
$\hat{\sigma}^2$	17.92	6.94	0.07	4.14	2.44	1.41
Broad sense heritability (h^2)	0.98	0.93	0.66	0.81	0.94	0.94

IRP				
Trait[a]	DRF	ARF2	APL2	AXL2
Unit	Days	#	cm	cm
Mean	14.5	5.4	10.2	4.6
Min–Max	8.5–26.8	3.1–16.8	3.9–19.6	1.3–10.8
Std Dev	3.6	1.8	3.1	1.8
Variance components				
$\hat{\sigma}^2_g$	15.59	1.13	17.96	2.36
$\hat{\sigma}^2_{ge}$	0.26	0.26	2.54	0.14
$\hat{\sigma}^2$	19.05	0.98	13.47	6.81
Broad sense heritability (h^2)	0.98	0.75	0.84	0.76

[a] See Table 1 for trait abbreviations

Fig. 3). LFN accession mean ranged from 9.7 to 41.5 rosette leaves per plant, with a survey mean of 22.5.

Few accessions had produced axillary inflorescences of scorable size (>0.3 cm) before their primary inflorescences had attained clipping size. Therefore, counts of axillary inflorescences at clipping (ACL) were usually zero, with a distribution skewed from 0 (many accessions) to 0.9 (9481; Fig. 1). Accession 9481 sporadically bore several paraclades of length equal to or greater than that of the primary inflorescence at the time of the latter's clipping. Precocious axillary development was observed in additional central Asian accessions (9481A, 9481b, 9481C and 9481D) as well as in some of western European origin (see Supplemental Data, Table I).

Clipping resulted in greater numbers of axillary inflorescences at re-flowering (ARF) in all 85 accessions (that is, ARF2 > ARF1; Fig. 2). Inter-accession variability within this trend is reflected in accessions' 12-fold range of ARF2:ARF1 ratios, from 1.2 (NFA-8) to 14.9 (Knox-18). ARF, APL (length of longest inflorescence) and AXL (mean length of all paraclades at re-flowering) are plastic responses, sensitive to the environmental influence of clipping and can therefore be informatively depicted as reaction norms (Pigliucci and Schlichting 1998) (Fig. 2). ARF and APL consistently increased and declined, respectively, across accessions as a result of clipping. AXL displays greater intraspecific diversity in response to clipping of the primary inflorescence (Fig. 2), with accessions' AXL1:AXL2 ratio ranging from 0.24 (Est-1) to 2.0 (Shadara).

ARF2 varied widely among accessions, from 3.1 (Wa-1) to 16.8 (Pu2-23) with a mean of 5.4. Unclipped plants display a lower and more limited range for ARF1, with accession mean ranging from 0.6 (RRS-7 and others) to 4.8 (Pu2-23) and an overall mean of 2.0 paraclades per unclipped plant at virtual re-flowering. The species-wide distribution of ARF1 values was mildly bi-modal, owing to a cluster of nine geographically diverse accessions (Tamm-27, Rmx-A02, Pu2-23, Se-0, Ts-5, NFA-8, Sapporo-0, Bur-0, Ren-11) creating a small shoulder at the upper end of the distribution (Fig. 1). This shoulder was absent in the ARF2 distribution, leaving one extreme outlier, Pu2-23 (ARF2 = 16.8).

DRF (days to re-flower after clipping) was measured only on unclipped plants. DRF means spanned a threefold range, from 8.5 (9481) to 26.8 days (Kz-1) with a survey mean of 14.5 days (Fig. 1).

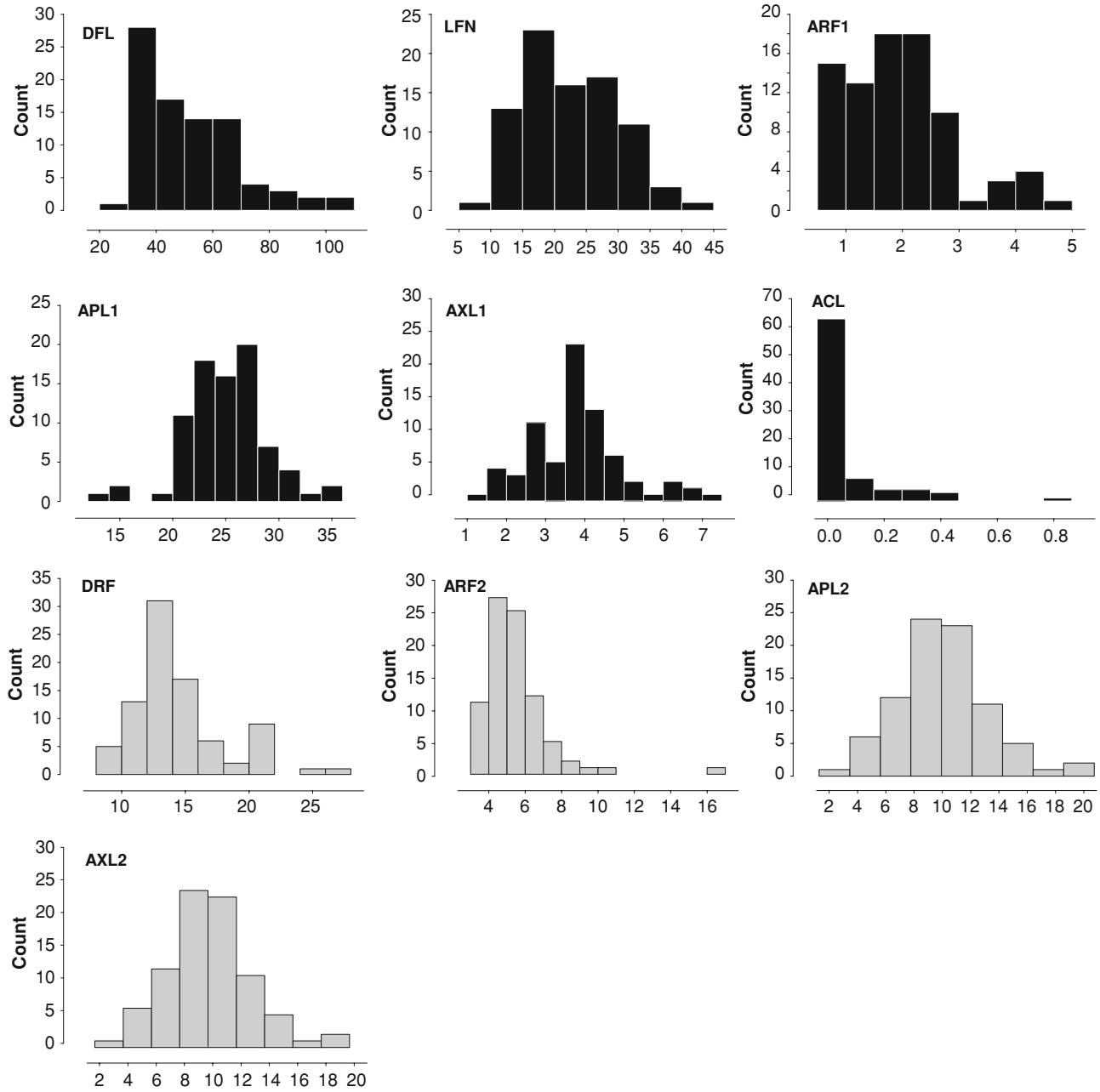

Fig. 1 Histograms of life history (*black*) and inflorescence replacement (IRP) (*gray*) traits for mean of 85 Arabidopsis accessions. *Vertical axes* of all plots are number of accessions. *Horizontal axes* are in units of trait. *Bars* in upper right of each plot represent standard deviations. See Table 1 for trait names, units, abbreviations and definitions

Pairwise correlations among traits

The 10 traits measured in this study are unlikely to be independently controlled at the genetic level. Pairwise correlations among the 10 traits were therefore analyzed to further define the phenotypic space represented by the 10 chosen IRP and life history characters (Olson and Miller 1958; Pigliucci 2007; Pigliucci and Preston 2004). Of the 45 possible pairwise correlations, 23 (14 positive, 9 negative) are highly significant ($p = 0.01$), with Pearson correlation coefficients ranging from -0.48 to $+0.91$ (Fig. 3). Four additional trait pairs are significantly correlated at the $p = 0.05$ level, all but one positively. The 45 comparisons can be subdivided into three categories with respect to the life history (LH) and IRP trait classes: 24 LH $\times$ IRP, 15 LH $\times$ LH and 6 IRP $\times$ IRP. Highly significant correlations

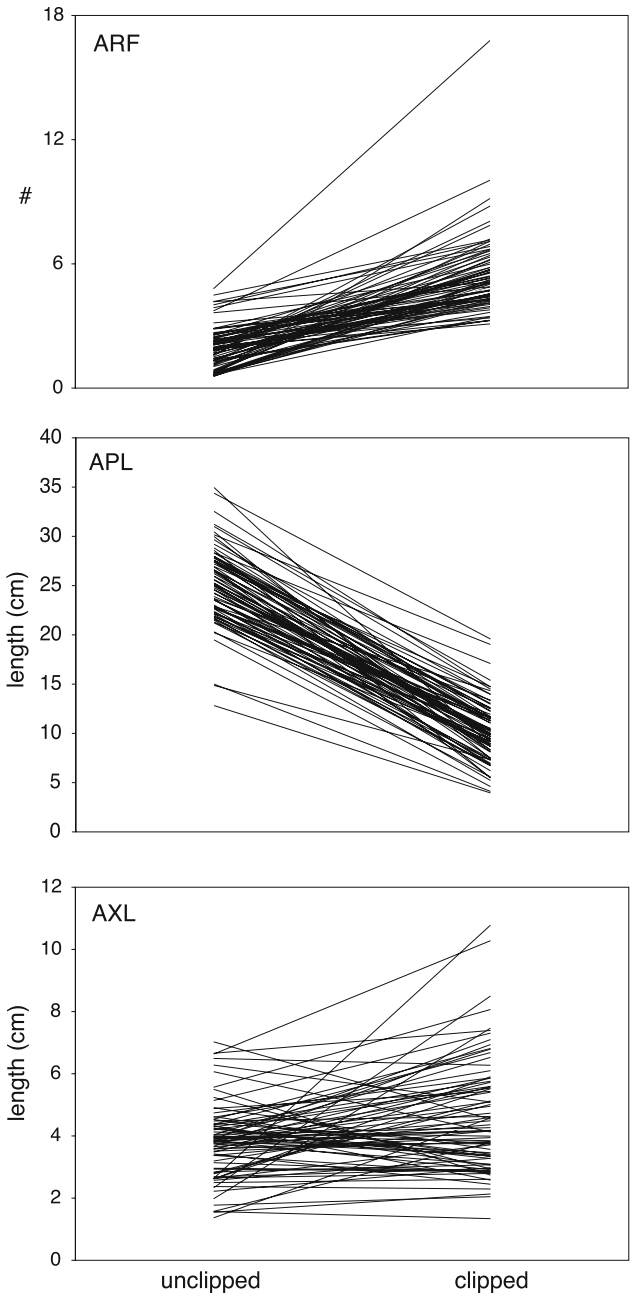

Fig. 2 Developmental reaction norms for ARF, APL and AXL traits in unclipped and clipped plants. Two points designated "unclipped" and "clipped" on *horizontal axis* represent ARF1 and ARF2, APL1 and APL2 and AXL1 and AXL2 for the top, middle and bottom plots, respectively

were roughly proportionately distributed among the three categories, with the majority (14 of 23) found in LH × IRP comparisons. Only one comparison of two IRP traits (APL2:AXL2, see "Discussion") was highly significant, with the remaining 8 highly significant correlations occurring within LH trait pairs.

Discussion

Natural variation for component traits of the Arabidopsis IRP is significant and heritable

Having identified significant intraspecific variation for all of the IRP component traits examined as well as for several life history characters, we reject the null hypothesis that no significant natural genetic variation exists for the inflorescence replacement program (IRP) in *Arabidopsis thaliana*. This finding is not unexpected for a species with as diverse an adaptive range as Arabidopsis (Blows and Hoffmann 2005).

Genetic dissection of a complex trait via the quantitative analysis of intraspecific variation begins with a species-wide survey of extant variation in the focal traits, with the breadth of germplasm examined being limited by the time and resources required to score phenotypes with adequate accuracy. Our examination of 85 accessions falls roughly in the middle of the published range of 15 (Juenger et al. 2000) to 188 (Perez Callejon et al. 1993) accessions for surveys of natural variation in Arabidopsis. Greater breadth and nuance of variation likely exists within a perfectly inclusive sampling of extant or extinct *A. thaliana* populations. Each accession examined here represents a single genotype drawn from a necessarily limited sampling of a standing Arabidopsis population from a discrete place and time. Given the levels of genetic diversity among and within populations of Arabidopsis (Bakker et al. 2006; Bergelson et al. 1998; Jorgensen et al. 1999), our survey must be viewed as incomplete on a truly species-wide basis.

We have partitioned the Arabidopsis IRP into discrete, measurable traits (Table 1) and plants were scored for IRP responses at re-flowering. Space limitations prohibited collection of data at plant maturity, such as those on higher order branching (Bennett and Cullimore 1990) and fitness. Absence of the latter precludes informed inferences regarding relative selective values of, and possible fitness trade-offs among, IRP phenotypes (Roff and Fairbairn 2007). Our primary near-term objective of gene discovery differentiates this study from more evolutionary and/or ecological inquiries regarding life history (Griffith et al. 2004; Mitchell-Olds 1996), herbivory and its consequences in Arabidopsis (Bidart-Bouzat 2004; Bidart-Bouzat et al. 2004; Ungerer et al. 2002; Weinig et al. 2003a, b).

Natural phenotypes, mutants and the model for AM activity

Mutants identified in conventional genetic screens frequently display extreme, maladaptive phenotypes, whereas

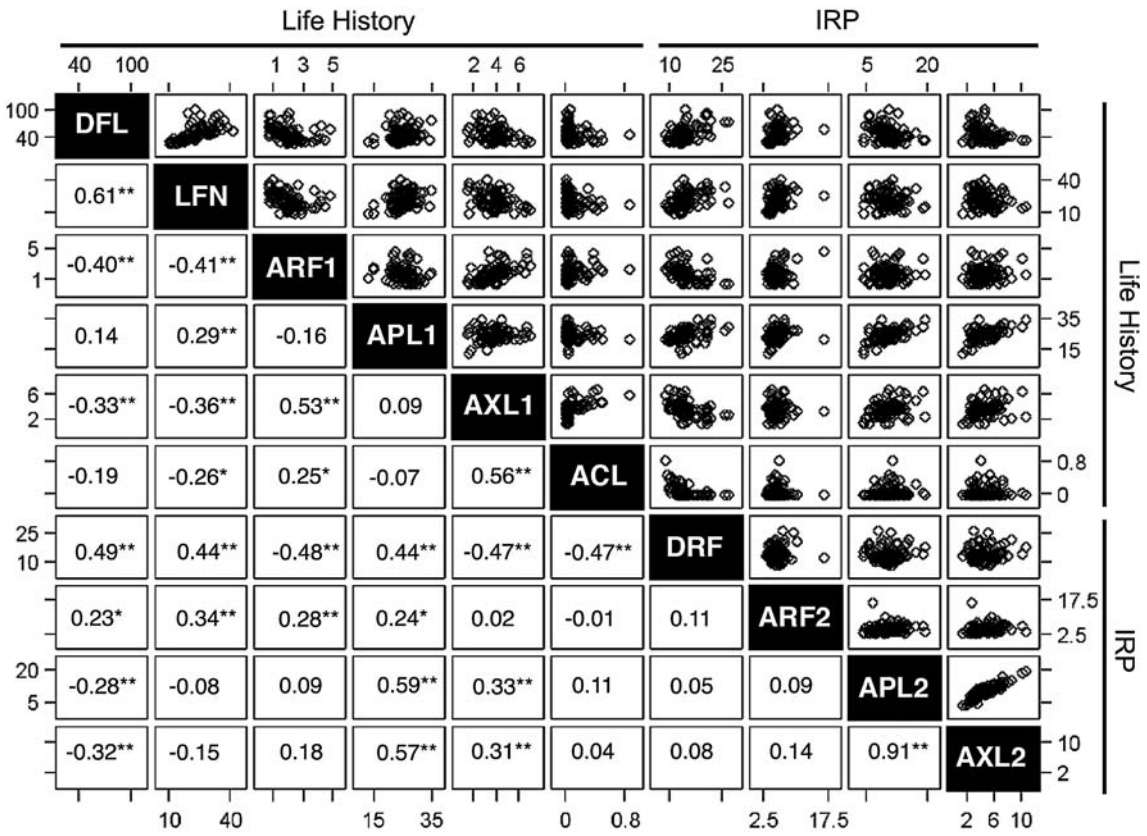

Fig. 3 Scatterplot matrix of 10 traits evaluated for 85 Arabidopsis accessions. *Diagonal* represents trait names as specified in Table 1. Above and right of diagonal are pairwise scatterplots of traits on the diagonal below and left of each plot. Below and left of diagonal are Pearson correlation coefficients for each pair of traits. ** and * represent trait correlations significant at $p = 0.01$ and $p = 0.05$ levels, respectively

natural variation exhibits a more subtle and continuous phenotypic spectrum vetted by natural selection. Orienting the variation documented in this study within the context of that portrayed in prior mutational studies of AM function may provide clues to underlying genetics.

The absence of amorphs or dramatic hypermorphs among the accessions surveyed suggests null mutations in genes controlling de novo AM formation, such as *LAX*, *BUSHY* or *REV* (Komatsu et al. 2003; Otsuga et al. 2001; Reintanz et al. 2001) are not represented among the germplasm surveyed. However, variation in APL, AXL or DRF could be mediated by allelic alternatives at these loci that delay or accelerate AM formation during development.

The Arabidopsis *max* mutants were recovered from screens for plants with "altered shoot branching" (Stirnberg et al. 2002), a phenotype reflected in ARF1 values reported here. *max1–max4* and *axr1–12* all show increased basal branching (unclipped) compared to wild-type (Bennett et al. 2006). Placing these within the continuum of our accessions' ARF1 values (Fig. 1) is problematic since ARF1 was scored long before the primary inflorescence had reached maturity, when reported *max* and *axr* branches were counted. Axillary inflorescence numbers ranged from 2 to 12 in *max* and *axr* single and double mutants (in a Col-0 background) at that stage (Bennett et al. 2006). Had our inflorescence counts been made closer to plants' senescence, then ARF1 values might well have extended upward, well beyond the 0.6–4.8 range reported here (Table 2, unclipped). While the clipping treatment elevated the ARF range's maximum to 16.8 (ARF2, Table 2), roughly comparable to that reported for *max* and *axr* mutants at maturity (Bennett et al. 2006), this range extended downward to 3.1, a value roughly comparable to both that of most unclipped plants in our study as well as the published value for unclipped wild-type Col-0 (Bennett et al. 2006). Thus, some Arabidopsis accessions appear to be naturally hypomorphic in their responses to removal of the primary inflorescence.

In light of a recent model proposed to accommodate data on auxin transport in *max* mutants of Arabidopsis, variation in the basal branching activity among genotypes could arise from variation in, among other factors, (1) levels of auxin supplied from apical sources, (2) auxin flux capacity through stem vasculature and buds, (3) sensitivity

and strength of functions along the signal transduction pathway (for example, MAX2 or AXR1), (4) sensitivity of buds to cytokinin, (6) capacity for biosynthesis and transport of MDS; and (7) the kinetics of establishing vascular connections to released AMs. While tests of some of these components await confirmation of strigolactone as the MDS and identification of its receptor, assays of polar auxin transport capacity in inflorescence internodes of accessions spanning the range of ARF1 values reported here would further test the proposed causal link between auxin transport capacity and AM activity (Bennett et al. 2006; Leyser 2006).

Accession 9481's axillary inflorescences frequently emerged prior to and soon out-grew their primary reproductive axes. Such axillary precociousness may arise from an anomaly in its auxin/cytokinin/MDS signaling path or from a cryptic defect in primary inflorescence development, perceived remotely by AMs as a condition indistinguishable from "damaged primary inflorescence". If such a defect exists, it is not manifest in the length of its primary inflorescence which, with an accession mean of 22.7 cm at re-flowering (APL1), falls in the center of the species-wide distribution.

Variation in length of paraclades at re-flowering (AXL and APL, Figs. 1 and 2) can be decomposed into: (1) Maturation distribution within the plant's axillary bud bank (Hempel and Feldman 1994; Leyser and Day 2003; Vesk and Westoby 2004); (2) Kinetics of bud break (Stafstrom 1993); (3) Rate of production of cells that populate the growing axes; and, (4) Cumulative, intrinsic elongation rate of the axes. Inter-accession differences in AM and bud maturation kinetics have not been documented in Arabidopsis, a seminal study having focused on a single accession, Landsberg *erecta* (Hempel and Feldman 1994). Cell production rates, coupled to levels of CDKA activity, contribute to inter-accession differences in rates of root elongation in Arabidopsis (Beemster et al. 2002). These are likely to be a factor in the kinetics of extension of aerial axes as well.

The overall rate of elongation of an axis is the product of internode production frequency [for example, *axr3* mutants, (Leyser et al. 1996)] and the aggregate of elemental axial expansion rates within internodes (Erickson and Sax 1956). Intraspecific variation in plastochron (time between leaf primordium initiation events) timing could mediate faster inflorescence elongation by generating new internodes (and thus more expanding cells) more frequently. However, inflorescence elongation rates (APL2/DRF) are poorly correlated ($r = -0.19$) with DFL/LFN quotients, the latter being a rough estimate of the average of all plastochrons during a plant's lifetime.

Studies of plants carrying mutations in the *DAD* and *HTD* genes of petunia and rice, respectively, revealed negative allometry between branch length and branch abundance (Napoli 1996; Napoli and Ruehle 1996; Nishikawa et al. 2005; Simons et al. 2007; Snowden and Napoli 2003; Zou et al. 2005). Such a relationship was not manifest in our sampling of Arabidopsis germplasm. The only significant correlation between paraclade number (ARF1 or ARF2) and heights (APL1, AXL1, APL2 and AXL2) in our study was a positive one between ARF1 and AXL1.

Implications of correlated life history and IRP traits

Several highly significant trait correlations occurred in trait pairs involving axillary inflorescence lengths in clipped plants versus unclipped plants (Fig. 3). These included APL1–APL2 (0.59**), APL1–AXL2 (0.57**), AXL1–APL2 (0.33**), AXL–AXL2 (0.31**) and APL2–AXL2 (0.91**) (Fig. 3), the latter being the strongest positive correlation observed between any two measured traits. APL2 values (length of longest axillary inflorescence at re-flowering) are, by definition, the largest values contributing to the mean represented by AXL2 (mean length of all axillary inflorescences at re-flowering). Such a strong positive correlation requires that APL2 values correlate just as strongly with the mean length of all other axillary inflorescences that emerge after clipping (i.e., $[(\text{ARF2} \times \text{AXL2}) - \text{APL2}]/[\text{ARF2} - 1]$). This is indeed the case ($r = 0.79$**). Thus, the length of the longest axillary inflorescence in a clipped plant at re-flowering reflects that of the other basal branches emerging with, or soon after, it. The analogous correlation in unclipped plants (APL1–AXL1) was notably not significant ($r = 0.09$). Therefore, absent removal of the primary inflorescence, Arabidopsis genotypes exhibit a wide range of primary inflorescence lengths at virtual re-flowering (APL1) for any given mean length of all axillary inflorescences (AXL1), and vice versa (Fig. 4). A strong positive correlation between APL1 and AXL1 would have suggested that the primary–axillary length relationship simply reflects a genotype's intrinsic axis elongation rate, shared by all inflorescence axes. The uncoupling of these traits in intact plants suggests that additional developmental or physiological factors govern the relationship between primary and axillary inflorescence elongation and that intraspecific variation exists for relative rates of elongation of basal axes in intact plants.

To what extent does the elongation rate of an inflorescence depend upon the amount of pre-existing vegetative biomass available to support its development? While rosette biomass was not explicitly measured in this study, LFN and DFL can be viewed as rough proxies for rosette size and age, respectively. Greater LFN and long DFL could in many cases result in greater photosynthetic

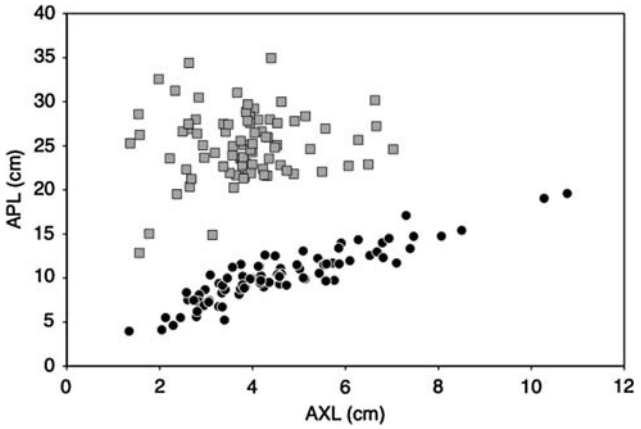

Fig. 4 Superimposed scatterplots of APL1 vs. AXL1 (unclipped, *gray squares*) and APL2 vs. AXL2 (clipped, *black dots*) illustrating change in relationship among inflorescences following clipping of primary inflorescence

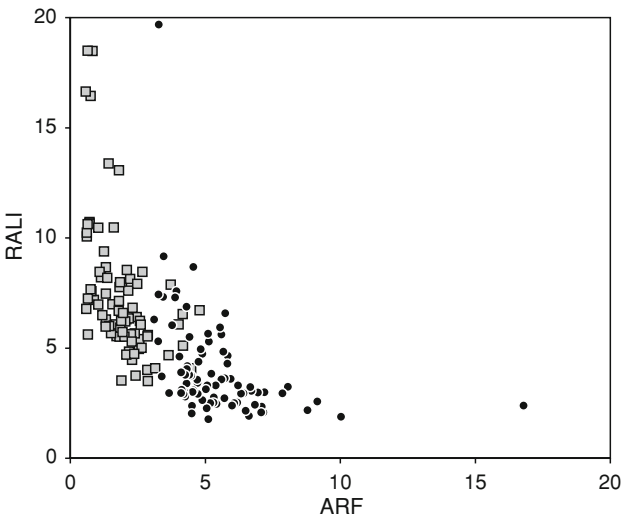

Fig. 5 Superimposed scatterplots of RALI vs. ARF for unclipped (*gray squares*) and clipped (*black dots*) plants showing shift in aggregate length–number relationship among inflorescences following clipping. See text for definition of RALI proportion

capacity and/or root system biomass. How does rosette size (LFN and DFL) relate to axis elongation rate (APL, AXL)? Of the eight relevant correlations, only LFN–APL1 are significantly positively correlated ($r = 0.29$**). Four others show significantly negative correlations (DFL–AXL1, LFN–AXL1, DFL–APL2 and DFL–AXL2), the remainder being non-significant. But static length is a weak proxy for cumulative elongation rate. The later can be derived as the quotient of APL2 and DRF, that is, final length divided by days elapsed since elongation began. Interestingly, the correlation between this calculated rate and LFN is also negative and highly significant ($r = –0.36$**), suggesting that leafier rosettes do not necessarily support faster-growing axillary inflorescences. Two possible rationales may underlie this inverse relationship. Maintenance of vegetative biomass and inflorescence growth compete for a common, limited pool of resources. Alternatively, Arabidopsis roots are the source of a carotenoid inhibitor of bud break (MDS) and older, more massive root systems may be more prolific sources of such a signal (Beveridge 2006; Leyser 2003).

Apical dominance is conventionally viewed in terms of the number, and less so the length, of axillary inflorescences. Nonetheless, heritable variation appears to exist among Arabidopsis accessions for the distribution of lengths of inflorescences that emerge, within a single plant, following decapitation. In order to compare such distributions among accessions, we calculated for each a value of within-plant axis length diversity at re-flowering and named this the relative axis length index (RALI). We define RALI to be the quotient of the length of the tallest inflorescence (APL1 or APL2) divided by the mean length of all other inflorescences [AXL1 for unclipped; $((ARF2 \times AXL2) - APL2)/(ARF2 - 1)$ for clipped]. Our sampling of accessions displays a strong negative correlation between ARF and RALI in both unclipped ($r = -0.52$**) and clipped ($r = -0.39$**) plants (Fig. 5). That is to say, the more axillary inflorescences a decapitated Arabidopsis plant produces, the narrower the distribution their lengths tend to be within a given plant. Thus, at re-flowering, the Arabidopsis accessions examined never bear many short axillary inflorescences dwarfed by a single overtopping floral axis.

Apical dominance and the response to inflorescence removal

Enumeration of axillary inflorescences (ARF1 and ARF2) more faithfully quantifies apical dominance sensu stricto than axis lengths at, or elongation rates prior to, re-flowering (Cline 1991). Given the developmental origin of axillary inflorescences, a simple "one leaf, one axillary meristem, one axillary inflorescence" hypothesis regarding ARF must be considered. Indeed, ARF2 and LFN are positively correlated ($r = 0.34$**), suggesting that leaf number plays at least a limiting and perhaps an enabling role in the quantitative response to clipping. Nevertheless, that the correlation is far from $r = 1.0$ suggests that additional factors impinge upon a leaf axil's or AM's destiny with respect to inflorescence emergence. Interestingly, unlike ARF2, ARF1 shows strong negative correlations with LFN ($r = -0.41$**) and DFL ($r = -0.40$**), indicating that, in unclipped plants, late flowering tends to attenuate paraclade development. As described above for APL and AXL, if we cautiously consider DFL a proxy for accumulated biomass, then we may observe a physiological link between DFL and ARF1. Despite their contrasting

correlations with LFN, the positive correlation between ARF2 and ARF1 ($r = 0.28$**) suggests that AM behavior in both clipped and unclipped plants is likely to be governed by at least some common underlying determinants.

Axillary inflorescence numbers that we recorded were obviously influenced by the time period our protocol allowed for these structures to develop to scorable size (>0.3 cm). However, the independence of ARF2 and DRF ($r = 0.11$) suggests that there is more to paraclade numbers in Arabidopsis than merely the time arbitrarily allotted for them to develop. In a similar vein, our choice of the day of re-flowering as that on which to enumerate axillary inflorescences may have biased relative inflorescence length measurements by arbitrarily tethering them to the program of floral maturation. However, DRF and APL2 were poorly correlated ($r = 0.05$), indicating that the time it takes an axis to bear an open flower is independent of its length. This finding echoes the observation that natural variation exists for axis elongation rates in Arabidopsis (Beemster and Baskin 1998; Mouchel et al. 2004; Ungerer et al. 2002), but adds the nuance that the kinetics of the floral maturation program are not coupled to the elongation rate of the axis that bears the flower.

DRF (days to re-flower) reflects the cumulative sum of the concatenated signaling events of bud break, axis elongation and floral maturation. It is particularly striking that DRF is highly correlated with every LH trait but with none of the IRP traits (Fig. 3). Two of DRF's LH correlations, with DFL ($r = 0.49$**) and LFN ($r = 0.44$**), warrant notice, although DRF's positive correlations with these are not statistically independent since DFL and LFN correlate positively with each other. The physiological basis for the DRF–DFL (LFN) correlation is obscure, but may again arise from the contribution of a larger root mass being a more prolific supplier of the branch-inhibitory signal, as alluded to above. The genetic basis for the conversion of the Arabidopsis shoot apical meristem from a vegetative to a reproductive state is well established (Boss et al. 2004), and a parallel developmental transition occurs in AM development concomitant with the floral transition. The signaling cascade ultimately driving the SAM from vegetative to reproductive mode also accelerates AM development in a basipetal fashion (Hempel and Feldman 1994). No mobile signal responsible for integrating development of AMs with that of a new floral SAM has been identified. Yet in this way, AM activity is functionally allied to that of the SAM through such signaling or ontogenic pleiotropy (or both), with the strong, species-wide DFL–DRF correlation possibly being a manifestation of such a connection. A selective advantage would accrue to a spring or fall annual that re-flowered quickly after inflorescence damage or loss. Such genotypes must carefully budget time during their single growing season to both vegetative and reproductive development. The biennial habit of winter annuals affords them a full growing season for reproduction. Acceleration of re-flowering in early flowering accessions would increase the likelihood of completing their life cycle in a single growing season.

The untapped bud bank: developmental constraint or conservative resource deployment?

The very existence of natural variation in axillary regrowth following decapitation in a monocarpic, annual plant raises the question, Why does not every rosette leaf's AM burst into reproductive growth immediately following loss of the primary inflorescence? What selective advantage is gained by such parsimonious conservation of reproductive potential? Two explanations are possible, one developmental and the other evolutionary. First, in contrast to lupine (Emery et al. 1998), not all AMs in Arabidopsis are developmentally competent to generate reproductive axes at the time of (our) clipping (Grbic and Bleecker 2000; Hempel and Feldman 1994; Long and Barton 2000). Hence, plants may well be making the most of what is available. Second, only one axis is lost in clipping of the primary inflorescence and one-for-one replacement is arguably a more prudent fitness preservation strategy than developing additional sinks in the form of possibly superfluous reproductive axes. That said, it should be noted that cauline bracts that flank basal paraclades in Arabidopsis form leafy whorls of varying complexity, each presumably energetically servicing the axis they subtend. A significant fitness advantage has been demonstrated in some plant species, *Ipomopsis aggregata* for example, that replace a single reproductive axis with a flush of axillary inflorescences following simulated or actual herbivory (Paige 1992, 1999).

Selective value of IRP variants

The intraspecific variation described in this report raises tantalizing questions as to its maintenance and role in the populations represented by surveyed accessions. Does the species-wide threefold range in DRF values represent authentic adaptations to challenges presented by herbivores with differing browsing habits? Unfortunately, our data are silent on such matters. Whereas our survey was broadly inclusive of available Arabidopsis germplasm, our sampling of populations was infinitely anecdotal: one inbred per population. How common or rare, adaptive or maladaptive, an accession's genotype was within the population from which it was drawn cannot be known from this work. Little is known about the provenance and habitat of origin of many publicly available Arabidopsis accessions to offer hints as to local selective forces (herbivore

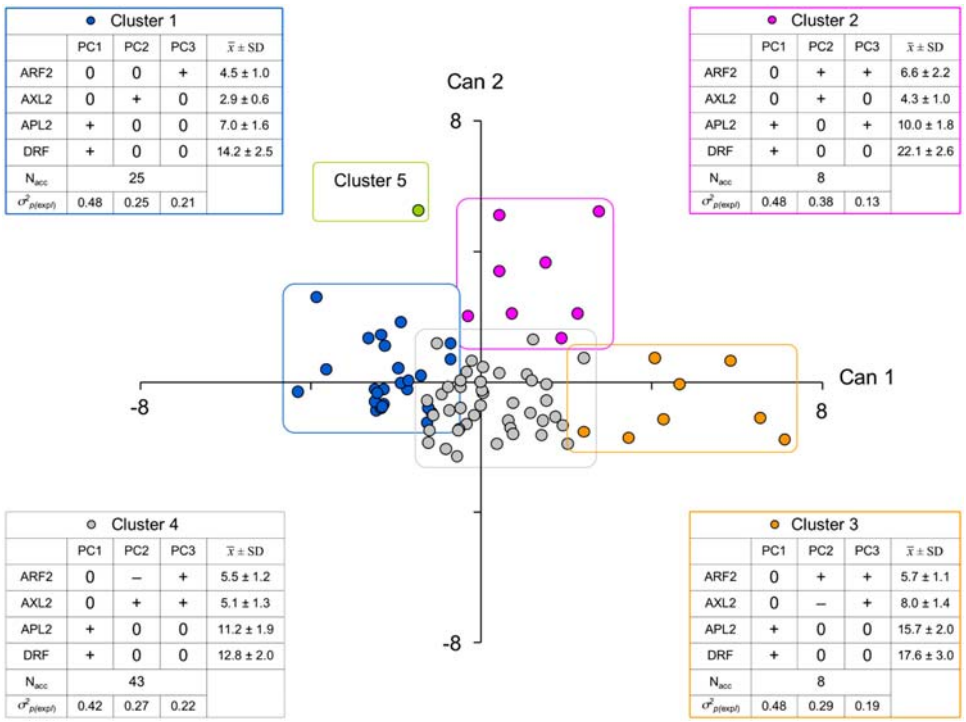

Fig. 6 Clustering of Arabidopsis accessions by IRP strategy. Clusters were identified by *k*-mean cluster analysis with a subsequent canonical analysis. Cluster 5 contains only one accession and is therefore not represented among the principal component (PC) analyses in the four corner tables. Each table shows number of accessions (N_{acc}) per cluster, cluster mean and standard deviation for each IRP trait ($\bar{x} \pm$ SD) and loadings of first three PCs within cluster (PC1, PC2 and PC3) where 0 = PC loadings $<|0.55|$, "+" = PC loadings >0.55, "−" = PC loadings <-0.55. All PC loadings >0.55 or <-0.55 were regarded as significant as suggested by Tabachnick and Fidell (2000). $\sigma^2_{p(expl)}$ is the proportion of phenotypic variance explained by PC1 to PC3, respectively. See Table 1 for description of traits and their abbreviations

behavior, wind, freezing or disease) to which these phenotypes may or may not represent evolved responses (Mitchell-Olds and Schmitt 2006; Tonsor et al. 2005).

We also do not know whether basal severing of 4-cm tall primary inflorescences (leaving the vegetative biomass of the rosette intact) is a reproductive challenge with which Arabidopsis has been presented consistently enough to have evolved a set of physiological and developmental responses. Rabbit and rodent herbivory is logically and widely presumed to be a selection pressure on Arabidopsis populations worldwide, and thus one to which the species has necessarily been obliged to respond evolutionarily (Weinig et al. 2003a). But specific behaviors of vertebrate browsers have not, to our knowledge, been documented with respect to this prey species as they have for others (Paige 1992).

Unpredictable developmental plasticity and genotype-by-environment interactions prevent us from knowing the degree to which an accession's measured performance in the growth room resembles that displayed in the natural setting of its origin, given that such environments differ from the growth room in every possible biotic and abiotic aspect imaginable. This point applies not only to the individual traits measured in this study, but also, it follows, to their covariances. Finally, all one can assert with reasonable certainty is that accessions' phenotypes observed in the growth room were produced by the same genomes that fashioned successful (i.e., fitness > 0) plants in the locales from which they were originally collected. How these genomes' manifested phenotypes resemble one another in the wild versus the growth room is unknown.

A discrete number of IRP implementations

Our introduction of the terminology "inflorescence replacement program (IRP)" implies that Arabidopsis expresses a suite of integrated phenotypes constituting a co-evolving response to inflorescence loss or damage (Berg 1960; Pigliucci 2007). If this is so, then the Arabidopsis genome should accommodate a finite diversity of implementations of such a program and accessions could therefore be categorized by IRP strategy. In order to begin exploring this conceptual framework of IRP phenotypic integration, we clustered the study's 85 subject accessions based on their BLUPs for the four IRP traits (Fig. 6). Optimization of clustering configurations resulted in a sorting of the germplasm into 5 clusters, each occupying a mutually exclusive domain in multidimensional *k*-mean

space (despite overlap when projected onto a two-dimensional coordinate system in Fig. 6). Whether the cluster represented by a solitary accession (Pu2-23, Cluster 5) is truly a novelty species-wide or simply the consequence of inadvertently skewed sampling cannot be answered at this time. Principal component (PC) analyses of the other four clusters reveal primary contributions from APL2 and DRF in defining the first PC of each cluster, accounting for 42–48% of their phenotypic variances. Whereas the loadings of the first PCs across clusters were comparable, clusters differed for their second and third PC loading patterns. Second PCs contributed 25–38% of σ_p^2 with loadings from ARF2 and AXL2, and a third adding another 13–22% of σ_p^2, with diverse loadings.

Inspection of IRP phenotypic clusters reveals no apparent geographical or molecular genetic affinity of accessions within them (Nordborg et al. 2005). Uncertainty regarding local biotic and abiotic features of accessions' habitats of origin precludes relating clusters to particular environments or selective conditions. Associations implied by the five clusters could arise non-selectively by genetic drift among dispersed, geographically and reproductively isolated populations, divergent natural selection among such populations in response to local conditions (Bergelson et al. 1998), or a combination of the two, but less likely (for these particular phenotypes) from larger scale geographic features (e.g., latitude, elevation) or post-Pleistocene familial descent (Sharbel et al. 2000).

Conclusion

This work establishes a foundation for future study of the Arabidopsis IRP. Our finding of significant, heritable, intraspecific variation in suitable metrics, coupled with a substantial database of nucleotide re-sequencing among most of the focal accessions (Nordborg et al. 2005), paves the way for a dissection of the Arabidopsis IRP's genetic architecture. Future research can address a number of enticing questions. Which chromosomal loci are responsible for the character variation and integration apparent worldwide in the Arabidopsis IRP? Are *MAX, PIN* or *BRC* among these loci, or will they be novel functions heretofore inaccessible to conventional forward genetic analysis? What is the molecular basis for within and between population IRP phenotypic variation and integration? How have these loci evolved alongside those governing other aspects (e.g., delayed senescence) of this and other species' post-herbivory recovery programs? How does the genetic architecture of the Arabidopsis IRP compare with that of other dicots? What constitutes the control and architecture of the inevitable phenotypic plasticities and genotype-by-environment interactions contributing to further variation in these traits and their associations? Are IRPs tailored by natural selection to local herbivore behaviors, or are such selective forces trumped by more ubiquitous abiotic factors? Can we identify a discrete set of IRP genetic programs worldwide, driven by a sort of intraspecific convergent evolution, or will we discover seemingly infinite underlying genetic novelty in plants' evolved strategies to accommodate this inevitable consequence of their trophic obligation to global ecosystems?

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