Effects of gibberellin mutations on tolerance to apical meristem damage in *Arabidopsis thaliana*

JA Banta and M Pigliucci

Department of Ecology & Evolution, 650 Life Sciences Bldg, SUNY-Stony Brook, Stony Brook, NY 11794, USA

To examine the role of gibberellin hormones (GAs) in tolerance to apical meristem damage (AMD), we characterized the reaction norms of several GA-deficient and insensitive mutants of *Arabidopsis thaliana* in response to AMD and compared them to those of the wild type, Landsberg, from which they were derived. We included 'natural' genotypes of *A. thaliana* – accessions with shorter lab histories – in order to evaluate how representative Landsberg is of other genotypes. The GA mutations did not alter the level of tolerance to AMD, which was consistent with equal compensation for all genotypes. Generally, the reaction norms to AMD did not differ among the GA mutants themselves, or between the GA mutants and Landsberg. The GA mutations did affect the overall phenotypes of the plants, but these effects were not simply related to whether the mutation was

early or late in the biochemical pathways. The GA-insensitive mutant was phenotypically different from the GA-deficient mutants and from Landsberg. The natural populations differed significantly from Landsberg, particularly in attributes related to size and inflorescence production, one more example of the need for researchers to be careful when generalizing the results of studies based upon laboratory strains. Our results indicate that early-flowering genotypes of *A. thaliana* can be remarkably tolerant to AMD, and that GA deficiency/insensitivity does not hinder tolerance to AMD, at least in this genetic background. Moreover, we confirm that mutations at regulatory loci can have noncatastrophic effects on fitness, as recently found by other investigators.

Heredity (2005) **94**, 229–236. doi:10.1038/sj.hdy.6800603
Published online 24 November 2004

Keywords: apical meristem damage; *Arabidopsis thaliana*; compensation; gibberellin mutants; tolerance

Introduction

The ecology and evolution of phenotypic plasticity is increasingly being studied from a combined organismal and genetic perspective (Scheiner, 1993; Pigliucci, 1996; Jackson *et al.*, 2002). This represents a significant step forward in comparison with the traditional study of plasticity based largely on quantitative genetic methods, which cannot investigate molecular pathways that induce plastic responses (Barton and Turelli, 1989; Pigliucci and Schlichting, 1997). Examples of this new hybrid approach include the examination of the reaction norms of known (and often molecularly characterized) mutants that affect the response to a specific environmental stimulus of interest (Schmitt *et al.*, 1995; Pigliucci and Schmitt, 2004). This allows us to see how individual loci that have been previously identified because of their specific phenotypic effects may help modulate a potentially adaptive plastic response.

In this paper, we examine plasticity to apical meristem damage (AMD) in an annual, monocarpic plant, hypothesizing that loci affecting the production of gibberellin (GA) hormones are important in mediating this plasticity. AMD can be caused by vertebrate herbivory among other environmental factors (Belsky, 1986; Paige and Whitham, 1987; Rosenthal and Kotanen,

1994; Juenger *et al.*, 2000; Tiffin, 2000). The damage releases apical dominance via a hormonal signal, allowing additional inflorescences to grow from the basal rosette (Paige and Whitham, 1987; Benner, 1988; Mopper *et al.*, 1991; Juenger *et al.*, 2000). The outcome of this release from apical dominance can be decreased fitness (undercompensation: Bergelson and Crawley, 1992; Huhta *et al.*, 2000a,b), little or no change in fitness (compensation: Maschinski and Whitham, 1989; Bergelson *et al.*, 1996; Juenger *et al.*, 2000), or, somewhat surprisingly, an increase in fitness (overcompensation: Paige and Whitham, 1987; Paige, 1999; Juenger *et al.*, 2000; Weinig *et al.*, 2003), depending upon how many additional inflorescence branches develop from the rosette after AMD and how many fruits are produced per branch.

In *Arabidopsis thaliana* (Brassicaceae), five GA mutants have been characterized and are available from the *Arabidopsis* seed stock center in Columbus, OH (www.arabidopsis.org). Four of these mutants are deficient in biosynthetic enzymes at different steps in the production of GAs (Koornneef and van der Veen, 1980; Kamiya and Garcia-Martinez, 1999), while the remaining mutant is GA insensitive, meaning that it produces GA but a key receptor is non-functional, so that the signal triggered by the hormones is not transduced (Koornneef *et al.*, 1985).

Our aim was to examine the reaction norms of GA mutants under conditions of apical damage or lack thereof in order to investigate if and how GA mediates the effects of AMD. We hypothesized that, in the wild type, GAs are involved in tolerance to AMD by promoting the elongation of proliferating inflorescence branches (Phillips *et al.*, 1995; Hedden and Phillips 2000,

Correspondence: JA Banta, Department of Ecology & Evolution, 650 Life Sciences Bldg., SUNY-Stony Brook, Stony Brook, NY 11794, USA;
E-mail: jbanta@life.bio.sunysb.edu
Received 1 April 2004; accepted 10 September 2004; published online 24 November 2004

Hay *et al.*, 2004) produced after AMD. GA mutants, on the other hand, have a short, bushy phenotype regardless of whether or not they are browsed (Ross, 1994; Ross *et al.*, 1997), since they either lack fully functional GAs or are unable to transduce the GA signal. The release from apical dominance that comes with being apically damaged should therefore not be advantageous in GA mutants, since the extra inflorescences produced cannot elongate sufficiently, nor, consequently, produce additional fruits. Therefore, we predicted that AMD would be more detrimental to GA mutants than to the wild type from which they were derived.

Our study included genotypes with either early- or later-acting GA point mutations. The later-stage mutants have less severe GA-deficient phenotypes (Koornneef and van der Veen, 1980; Koornneef *et al.*, 1985; Zeevaart and Talon, 1992), and should be more phenotypically similar to the wild type, for example, by more effectively elongating the replacement inflorescences. Therefore, we also predicted that later-acting mutants should show more tolerance to AMD than earlier-acting mutants.

We compared the five GA mutants to the nonmutant (wild type) background genotype from which they were derived, Landsberg *erecta* (Ler-2), which is a laboratory line maintained for decades under controlled conditions. We also included in our experiment three other early-flowering genotypes of *A. thaliana* that have been maintained under laboratory conditions for a much shorter period of time (a few years); these populations are presumably more representative of natural (nonlaboratory) genotypes. We included these accessions in order to evaluate how other *A. thaliana* genotypes respond to AMD. If Ler-2 and these other genotypes react similarly to AMD, then it is plausible to infer that a GA mutation would have similar effects on tolerance in these other genetic backgrounds. On the other hand, it is possible that laboratory lines behave in a highly unusual manner, which should caution evolutionary ecologists from generalizing results based on standard laboratory stocks.

The reason for using only early-flowering genotypes in our study is that early- and late-flowering genotypes of *A. thaliana* are known to be different genetically and ecologically (Zhang and Lechowicz, 1994; Donohue, 2002), and are subject to distinct selection regimes. Therefore, a comparison between early-flowering Landsberg and late-flowering genotypes would not be informative in this context.

Our premises can be summarized in the following questions and expectations: (1) Do mutations in GA signaling alter the tolerance of *A. thaliana* to AMD? We predicted that the GA mutants would be more detrimentally affected by AMD than the wild type. (2) Do the effects of AMD differ for GA mutants deficient at different loci, and are these effects different from those of the GA-insensitive mutant? We expected the later-acting mutants to display a stronger response to AMD than the early-acting or the insensitive mutants (the latter expected to be phenotypically similar to some of the deficient mutants, despite the different genetic basis of its phenotype). (3) Does Ler-2 respond differently to AMD than more natural early-flowering genotypes? Although we did not have strong *a priori* expectations here, it was important to test how much of a good model standard stocks, such as Landsberg, actually are at providing us with generalizations about *A. thaliana*.

Materials and methods

Plant material

A. thaliana is a small, short-lived, highly selfing (Abbott and Gomes, 1989), annual monocarpic plant characteristic of ruderal habitats (Napp-Zinn, 1985). Over the past several years it has been used by an increasing number of evolutionary biologists as a model system (Pigliucci and Schlichting, 1995; Stahl *et al.*, 1999; Dorn *et al.*, 2000; Jackson *et al.*, 2002) because of its logistic advantages as well as the availability of a large knowledge base on its physiology, development, and molecular biology (Pang and Meyerowitz, 1987; Anderson and Roberts, 1998). *Arabidopsis*' annual life history simplifies the measurement of fitness components and how they are affected by genetic variation and environmental factors. In particular, fruit production at the time of senescence is a good estimate of total lifetime fitness (Westerman, 1970), since there is no allocation to vegetative structures for future seasons.

We used nine single-seed descent genotypes of *A. thaliana* from the Arabidopsis Resource Center (Columbus, OH, USA; www.arabidopsis.org): Landsberg *erecta* (Ler-2, Germany); Eilenburg (Eil-0, Germany); Wilna (Wil-2, Russia); Blanes (Bla-12, Spain); *ga1-5* (gibberellin mutant, hereafter referred to as GA); *ga1-6* (GA mutant); *ga4-1* (GA mutant); *ga5-1* (GA mutant); *gai-1* (GA mutant). The three early-flowering genotypes Eil-0, Wil-2, and Bla-12 were picked at random from a set of genotypes previously used by Pigliucci and Marlow (2001). The GA mutants were chosen as part of a broader research program on the reaction norms of hormonal mutants in *Arabidopsis* (Pigliucci and Schmitt, 2004). Ler-2 was included for comparative purposes, since the five GA mutants were originally derived from it. All of our comparisons were either between Ler-2 and the GA mutants, to see how the mutants responded relative to their genetic background, or between Ler-2 and the natural accessions, to see how Ler-2 responded relative to other early-flowering genotypes.

Four of the five mutants examined in this study are GA-deficient, that is, the mutation precludes the formation and accumulation of GAs in the plant. As a consequence, under normal conditions, all these mutants are dwarf or semidwarf, a characteristic of GA-deficient phenotypes consisting of a bushy appearance and a decreased height (Koornneef and van der Veen, 1980; Ross, 1994; Ross *et al.*, 1997). Two mutants, *ga1-5* and *ga1-6*, are allelic (Koornneef and van der Veen, 1980; Zeevaart and Talon, 1992), and act very early in the GA production pathways (Ross, 1994; Ross *et al.*, 1997). The remaining two gibberellin-deficient lines (*ga4-1*, and *ga5-1*) have a similar general phenotype (Koornneef and van der Veen, 1980), but map to distinct genetic loci (Koornneef and van der Veen, 1980; Zeevaart and Talon, 1992); *ga5* appears to be blocked at several different intermediate steps in the pathways, and *ga4* is interrupted at a still later level as compared to *ga1* (Ross *et al.*, 1997). *ga1-5* and *ga1-6* are dwarfs, whereas *ga4-1* and *ga5-1* are classified as semidwarfs (Koornneef and van der Veen, 1980; Ross, 1994; Ross *et al.*, 1997). The reason for the differences in the severity of the GA-deficient phenotype between the early-acting (*ga1-5* and *ga1-6*) and the late-acting (*ga4-1* and *ga5-1*) mutants is not clear. All four of these

GA-deficient mutants are suspected to be 'leaky,' that is, they produce some small quantities of active GA, presumably due to redundancy in the GA-production pathways (Koornneef and van der Veen, 1980; Koornneef *et al*, 1985; Zeevaart and Talon, 1992; Phillips *et al*, 1995; Hedden and Phillips, 2000).

In contrast to all of these, *gai-1* is a GA-insensitive line, although it retains a low level of sensitivity to externally applied GA (Koornneef *et al*, 1985). Bioactive GAs are produced and accumulate in the plant's tissues, but are ineffective, possibly because the mutation damaged a key transcription factor in the transduction pathways (Koornneef *et al*, 1985). This mutant phenotypically resembles GA-deficient genotypes (dwarf appearance), although the genetic and physiological bases of the defect are entirely different (Koornneef *et al*, 1985).

Plant handling and experimental set-up

Seeds of all lines were imbibed with water and exposed to a brief cold treatment (9 days) at 4°C in the dark to facilitate germination. They were then planted in 4 by 4 by 4.5 cm pots with standard promix potting soil that had been autoclaved to avoid pathogen growth. Plants were housed indoors in three-level racks, and the photoperiod was set at 16 h of light per day at room temperature. The 9 days of cold treatment, followed by planting, were carried out on different schedules (because of logistical limitations). We therefore used planting date, which can be thought of as a temporal blocking effect, as a cofactor in the analyses.

The experiment included the nine genotypes exposed to two AMD treatments in a fully factorial design. There were three blocks, originally with 288 plants in each block; however, some of the soil accidentally dried up, leaving 192 plants surviving in block 2. Taking into account the plants lost to desiccation and to random mortality, the number of replicates for each genotype-treatment combination averaged four, and the total number of plants in the experiment was 640.

Nutrients, in the form of Osmocote time release pellets, were added 1 week after germination. The plants were either clipped to apically damage the plant's main inflorescence, or were left unclipped. Clipping was carried out at the time of bolting, when the inflorescences were in the unopened flower bud stage. The entire inflorescence was clipped off at the base of the rosette with scissors (although no rosette leaves were damaged or removed). This type of AMD mimics the damage done by mammals such as rabbits and deer, and has been observed in field experiments with *A. thaliana* (Weinig *et al*, 2003) and other monocarpic annuals (Paige and Whitham, 1987; Paige, 1994, 1999, Lennartsson *et al*, 1997).

The following traits, potentially affected by AMD, were measured: (1) Number of basal inflorescences, or inflorescences growing out of the rosette. This is a component of plant architecture during the reproductive phase, and a measure of how much basal inflorescence proliferation occurs when apical dominance is released. (2) Number of lateral stems (or branches), a second measure of plant architecture providing a quantification of the degree of bushiness of the main body of the plant, since lateral stems branch off from the basal inflorescences. (3) Fruit production, an estimate of lifetime

reproductive fitness. (4) Inflorescence weight, another estimate of reproductive fitness; we weighed all inflorescences growing on the basal and lateral branches for this measurement. (5) Seed germinability, also a component of fitness. In order to examine this, we gathered between 20 and 40 seeds from a subset of plants after senescence, put them in cold treatment for 5 days, and then left them at room temperature in a greenhouse for 10 days. We scored the proportion of seeds that germinated from each plant, and used this as our measure of seed quality.

Data analysis

We analyzed the data with a two-way analysis of variance (ANOVA). We checked for assumptions of normality and heteroscedasticity, and found that the residuals from the models for inflorescence weight and lateral branch number were truncated around the mean in the frequency distribution. We therefore transformed the data by taking the logarithm of the trait values (Sokal and Rohlf, 1981); the residuals of the reanalyzed data were then normally distributed. Analyses were carried out with Jump-In (SAS) version 4.0.4 using a General Linear Model approach (JMPIN, 2001). For each trait, the full model included the following main effects and interactions: genotype (quantifying genetic differences among lines; treated as a fixed effect); AMD (overall plasticity to clipped *vs* unclipped treatments; fixed effect); block (microenvironmental effects; random); planting date (covariate; random); AMD by genotype interaction effect (genetic variation for plasticity to AMD); and error (residual variance). We did not perform sequential Bonferroni or similar corrections for multiple simultaneous tests, following the advice of Moran (2003). Rather, we report the formal *P*-values, effect sizes (in the form of mean sums of squares), and the power of the tests (Cohen, 1992), and discuss the results from a statistically conservative perspective.

For effects that were found to be not statistically significant, we performed power analyses using G-Power (Buchner *et al*, 1997). We followed the conventions of Cohen (1992) and evaluated whether we had the statistical power to detect 'medium' and 'small' effects caused by our treatments. 'Medium' effect size (ES) means that treatment differences are 'visible to the naked eye of a careful observer' (for example, the clipped plants have visibly more fruits than the unclipped plants), whereas 'small' ES means that the treatment differences are 'noticeably smaller than the medium but not so small as to be trivial' (for example, the clipped plants have more fruits than the unclipped plants, but this is not as obvious; Cohen, 1992). This analysis accounts for the possibility that we found an effect to be statistically nonsignificant, not because the effect is actually biologically insignificant, but rather because of a limited sample size. Power values range from 0 to 1, and are calculated for each effect in the model based upon the degrees of freedom and the ES (small, medium, or large) of interest. Power values of 0.8 and higher are considered to be sufficient in order to conclude that there was enough power to detect an effect of the size of interest (Cohen 1992). We will refer to power values for medium ES as 'PW_{med}' and power values for small ES as 'PW_{small}'.

In order to examine the multivariate relationships among traits (ie, taking into account possible intertrait correlations), we performed a principal components analysis (PCA) on all characters (inflorescence weight, lateral branch number, basal inflorescence number, and fruit number), except seed germinability, since we used only a subset of plants to measure that trait. We standardized the data for each character around a mean of zero, and performed a PCA on the covariance matrix (Dillon and Goldstein, 1984, p 36). Plots in principal components (PCs) space allowed us to explore the phenotypic similarity of plants as a function of their genotype and AMD treatment. The PCA was carried out using Jump-In (SAS) version 4.0.4 (JMPIN, 2001).

Results

The main effects of genotype, block, and planting date were significant for all traits (inflorescence weight, number of lateral branches, number of basal inflorescences, fruit production, and seed germinability: Table 1). AMD was not statistically significant for any of the traits except for basal inflorescence number (Table 1), even though we had sufficient power to detect an effect of medium magnitude (all traits, $PW_{med} > 0.99$); we also had relatively good power to detect an AMD effect of small magnitude for all traits except seed germinability ($PW_{small} = 0.71$; for seed germinability, $PW_{small} = 0.61$). Furthermore, for all characters the percentage of variance explained by AMD was in fact very low (less than 1%),

indicating little biological relevance regardless of statistical considerations.

In general, mean sums of squares showed that statistically significant main effects accounted for 1–18% of the total variance. There were no significant interaction effects (Table 1), even though we had sufficient power to detect interaction effects of medium magnitude ($PW_{med} > 0.96$ for all traits). We lacked sufficient power to detect interaction effects of small magnitude, as is usually the case for higher-order effects in ANOVAs of large experimental designs. At any rate, mean sums of squares indicate that most interaction effects explained very little of the total phenotypic variance, ranging from 0.5 to 1.8%.

We examined the genotypic means and their 95% confidence intervals for all (across-environment) mean trait values. As an example, we show the plot only for basal branch production (Figure 1), but report the full genotype-by-treatment data in the Supplementary Table 1. In what follows, we focus on comparing the wild-type Ler-2 to its GA mutants, as well as Ler-2 to the other natural populations. We do not stress comparisons of the GA mutants to the non-Landsberg lines, as this would make little biological sense.

As far as the production of basal inflorescences is concerned, the natural genotype Bla-12 had slightly more basal inflorescences than the other natural accessions (Figure 1). The mutants *ga1-6* and *gai-1* had more inflorescences compared to the other mutants and to the wild-type Ler-2. It is worth noting that the only significant AMD main effect was on this trait, with

Table 1 ANOVAs for each trait (columns) and factor (rows) in the experiment

		Inflorescence weight (g)	Lateral branch number	Basal inflorescence number	Fruit number	Seed germinability
R^2		0.17	0.25	0.30	0.33	0.23
Genotype (G)	df	8	8	8	8	8
	MS	0.016	4429.50	6.36	530157.66	2.19
	MS%	6.64	13.77	15.83	9.86	9.21
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AMD (A)	df	1	1	1	1	1
	MS	~0	9.32	0.23	7492.00	~0
	MS%	0.09	0.03	0.56	0.14	0.01
	P	0.4110	0.6270	0.0312	0.2587	0.7795
A × G	df	8	8	8	8	8
	MS	0.004	170.63	0.35	27776.76	0.17
	MS%	1.75	0.53	0.88	0.52	0.73
	P	0.1157	0.8259	0.5039	0.7845	0.8251
Block	df	2	2	2	2	2
	MS	0.008	651.39	0.36	266331.44	1.37
	MS%	3.05	2.02	0.90	4.95	5.77
	P	<0.0001	0.0003	0.0243	<0.0001	<0.0001
Planting date	df	11	11	11	11	11
	MS	0.017	3017.64	3.47	986888.22	1.06
	MS%	6.74	9.38	8.65	18.36	4.47
	P	<0.0001	<0.0001	<0.0001	<0.0001	0.0067
Residual	df	605	606	606	607	471
	MS	0.202	23896.46	29.40	3557457.77	19.01
	MS%	81.72	74.27	73.17	66.17	79.80

For each factor, we report the degrees of freedom (df), mean squares (MS), MS as a percentage of the total, and *P*-values. Statistically significant effects at the $P < 0.05$ α value are highlighted in boldface. R^2 refers to the amount of variance explained by the whole model for a particular trait.

clipped plants having slightly more basal inflorescences than unclipped plants (unclipped 5.78 (confidence interval: 3.27 to 4.35), clipped 6.28 (3.65 to 4.85)), as would be expected with the release from apical dominance by clipping.

Concerning the other traits (plots not shown), the natural genotype Wil-2 had a lower across-environment mean inflorescence weight than the other two natural accessions, Eil-0 and Bla-12, while the GA mutants and Ler-2 had similar inflorescence weights. Bla-12 and Eil-0 produced more lateral branches than Ler-2 or Wil-2, but the lateral branch production of Ler-2 and the GA mutants were similar. With regards to fruit production, the significant genotypic variation in the ANOVA was mostly due to the greater trait mean of *gai-1*, with the other genotypes showing similar levels of fruit production to one another. It is interesting to note that the GA mutants produced as many fruits, or more, than the Ler-2 wild type, and that the latter produced roughly as many fruits as the other natural accessions. Finally, the

GA mutants did not differ from Ler-2 in seed germinability (60–70%), and Ler-2 in turn did not differ from the natural genotypes, with the exception of Eil-0, which was characterized by a higher value (about 87%, as compared to 73% for Ler-2).

In our study, no genotypes showed instances of overcompensation or undercompensation, as indicated by the lack of a main AMD effect – or an AMD by genotype interaction effect – for fruit number or seed quality, even though we had the statistical power to detect such effects of medium magnitude or smaller.

In the multivariate analysis, PC 1, which accounted for 66.6% of the variance, was mostly influenced by plant size, since all traits had roughly equal and positive loadings on that component (number of lateral branches = +0.54; fruit production = +0.52; inflorescence weight = +0.51; number of basal branches = +0.42). PC 2, which accounted for 16.7% of the variance, was influenced largely by basal branch number, given that this trait had a loading on that component that was much larger than those of the other characters (+0.87, as compared to −0.40, which was the next largest value). PC 3 accounted for an additional 8.8% of the total variance, and was influenced by inflorescence weight and fruit production (loadings: inflorescence weight = +0.70; fruit production = −0.70; next largest value = 0.10). It is also interesting to note that the magnitudes of the loadings of inflorescence weight and fruit production on PC 3 were opposite, suggesting a possible weak trade-off between these fitness components.

In the plot of PC 1 vs PC 2 (Figure 2), there was generally no difference between the clipped and the unclipped plants of each genotype, with the exception of *gai-1* and *gai-6*, both of which produced more basal inflorescences when clipped as compared to when not clipped, and Eil-0, which produced more basal inflorescences when unclipped compared to when clipped. *gai-1* and Eil-0 were larger when clipped, whereas *gai-6* was smaller. Ler-2 clustered near some of the GA

Figure 1 Least-squares (across-environment) means of basal inflorescence production for the different genotypes. The bars represent ± 1 SE.

Figure 2 PCA showing how the different genotype-AMD combinations cluster in multivariate space. Fruit production, inflorescence weight, number of basal branches, and number of lateral branches were included in the analysis. The black lines are the unclipped treatment and the gray lines are the clipped treatment. The bars represent ± 1 SE around the centroid means.

mutants (*ga1-5*, *ga4-1* in the unclipped state, and *ga5-1*), but was phenotypically distant from *gai-1*, which was much larger, and *ga1-6*, which was larger than Ler-2 in the unclipped state and had more basal inflorescences than Ler-2 in the clipped state. Ler-2 grouped distinctly from all of the natural accessions, which by comparison were characterized by increased size and proliferation of basal inflorescences (Figure 2).

Discussion

Researchers have insistently been calling for an integration of modern developmental and molecular biology into evolutionary and ecological studies (eg, Schlichting and Smith, 2002). This synthesis is ongoing in the case of phenotypic plasticity (Pigliucci, 2001), particularly concerning the genetic bases of plastic responses (Scheiner, 1993; Pigliucci, 1996; Crews, 2003). Since different molecular mechanisms can underlie a given pattern of phenotypic plasticity, it is necessary to directly integrate the molecular level of analysis in our studies to help us discriminate between alternative hypotheses concerning how a particular plastic response is actuated.

Our research combines information from molecular and organismal biology by examining the patterns of an ecologically relevant plastic response in a set of mutant and natural genotypes. Our aim was to determine whether specific mutations of known molecular underpinning, conferring GA deficiency or insensitivity, alter the level of tolerance of *A. thaliana* to AMD. This would suggest the potential for a role of GA in the evolution of tolerance to AMD, although of course not necessarily through the specific mutations examined here.

Effects of GA deficiency and insensitivity on fruit production and tolerance

The GA mutants included in this study did as well as Ler-2 in terms of tolerating AMD, implying that GA deficiency/insensitivity is not a handicap in terms of maintaining reproductive fitness following AMD. The fact that lack of GA did not seem to have an effect on tolerance is interesting, considering that GA is a major plant hormone known to have complex consequences for meristematic growth. In fact, GA deficiency both increases the number of basal meristems that elongate (thereby giving the plant a more 'bushy' phenotype), and reduces the extent of these meristems' growth (yielding a shorter, more compact, appearance) (Hay *et al*, 2004). Our results indicate that both Ler-2 and the GA mutants show little plasticity to simulated herbivory, which implies that GAs have little to do with the slope of the reaction norm for fruit production in response to AMD.

In addition to not affecting tolerance to AMD, these major regulatory mutations also had little or no apparent detrimental effect on fruit production, regardless of the treatment. In fact, the mutant *gai-1* even had higher fruit production than Ler-2. This surprising outcome may be explained by the double effect of GA deficiency on meristem activity (which is enhanced) and growth (which is diminished) mentioned above: if these two outcomes on average simply balance each other out in the mutants, then the overall fruit production will be similar to that of the wild type. Low fitness was not manifested in lower seed quality, either: the mean seed

germinability of the mutants was no different from that of Ler-2. These results are consistent with the work of Pigliucci and Schmitt (2004), who found that the same GA mutants did as well as or better than Ler-2, in terms of reproductive fitness, under greenhouse conditions. Our study provides more evidence (see, for eg, Purugganan and Suddith, 1998) that evolution could proceed by the spread of mutations at major regulatory loci: they are not necessarily hindered by strong deleterious pleiotropic effects; our mutants still flowered and set fruit, producing seeds of as good a quality as the wild type. Of course, how much all of this applies to field conditions is an open question, although mutations at regulatory loci are known to persist in wild populations of *Arabidopsis* (Aukerman *et al*, 1997).

We found no significant main effect of AMD and no AMD by genotype interaction in the statistical model explaining fruit production, even though we had the power to detect such effects. Therefore, we conclude that there were no positive or detrimental effects of AMD on reproductive fitness, and that genotypes showed equal compensation at all nutrient levels. This is important, because it implies that all genotypes were able to recover from the removal of the main inflorescence right before flowering, when compared to the situation with an intact apical meristem. Our results are consistent with the findings of Juenger *et al* (2000) in their simulated vertebrate herbivory experiment examining early- and late-flowering populations of *Gentianella campestris*, in which they found that early-flowering populations show equal compensation to simulated herbivory, whereas late-flowering populations display genetic variation for tolerance (although the mechanism accounting for this is currently unknown).

Since the genotypes included in our study all showed compensation, we conclude that the unclipped plants had some potential to produce fruits, which was not realized unless the apical meristems were in fact cut off. One possible explanation for this is that the main inflorescence was acting as an 'herbivory monitor' of when the herbivores leave and when it is opportune to resume the reproductive phase (Van Der Meijden, 1990). Such adaptive explanations, of course, warrant much more empirical investigation before they can be accepted as likely.

The multivariate picture

The principal components analysis clearly separated Ler-2 from the other natural accessions. This is in accordance with what is known about Ler-2's diminutive phenotype relative to other *A. thaliana* genotypes. Although our study involves only one laboratory strain (and does not include other often-used laboratory lines, such as Columbia), these results should at least caution against automatically assuming that long-established laboratory lines are representative of the species of interest. Indeed, results from other studies have sparked quite a debate over the appropriateness of using laboratory-adapted organisms to draw conclusions about the ecology and evolution of model species (eg, Matos *et al*, 2000; Sgrò and Partridge, 2000). The fact that Ler-2 is phenotypically distinct from the natural accessions may imply that the effects of GA mutations in a Ler-2 background are different from their effects in other, more typical, genetic

backgrounds. Therefore, although our study does not indicate a role of GA's in tolerance to AMD, these results should be interpreted with the caveat that the wild type was itself rather atypical; GAs might mediate tolerance in other genetic backgrounds.

We also found that most of the GA-deficient mutants grouped near Ler-2 in multivariate phenotypic space, showing that a clear effect of their common genetic background was still evident in the overall phenotype of the mutants. The phenotype of *gai-1*, however, was appreciably different from Ler-2 and the other mutants, particularly in terms of overall plant size. It is not clear why the only GA-insensitive mutant in our sample should have a markedly distinct phenotype, given that we expected the genetic defect to produce phenotypic effects analogous to those of GA-damaged pathways. However, even among the GA-deficient mutants there were detectable phenotypic differences, although these did not seem to be generally related to whether the mutations were affecting the GA pathways early or late. Indeed, even *gal-5* and *gal-6*, which are at the same locus, mapped in different areas of principal components space, possibly due to different degrees of 'leakiness' of their respective allelic mutations.

Concluding remarks

Overall, our results do not demonstrate a role of GA in mediating tolerance to AMD, although GA deficiency and insensitivity do seem to have an effect on other aspects of the phenotype. A role for GA in tolerance to AMD, however, might exist in other genetic backgrounds, especially late-flowering genotypes of *A. thaliana*, which differ dramatically both ecologically and genetically from earlier-flowering accessions (Zhang and Lechowicz, 1994; Juenger *et al.*, 2000; Donohue, 2002; Weinig *et al.*, 2003). As mentioned earlier, in *G. campestris*, later-flowering populations do show more genetic variation for tolerance than earlier-flowering accessions (Juenger *et al.*, 2000), although the mechanistic reasons for this are largely unexplored. Furthermore, in *A. thaliana*, Zhang and Lechowicz (1994) found that there was more phenotypic plasticity in many morphological and physiological traits in later-flowering genotypes than in earlier-flowering ones. Therefore, it is possible that GA becomes more important in mediating responses to AMD in later-flowering populations characterized by a longer life span and different ecological settings.

Acknowledgements

We thank Lori Cooke, Warren Denning, Ekaterina Shevchenko, and Allison Tanguay for their help with this experiment. We also thank Lisa Dorn, Zachary German, Kelly Gravuer, Nolan Kane, David Mooney, Ken Paige, Johanna Schmitt, Yuko Toyonaga, and Cynthia Weinig for their helpful discussions on this topic. This research was partially supported by NSF Grant DEB-0089493 to MP and by a University of Tennessee Department of Botany Research Enhancement Award to JB.

References

- Abbott RJ, Gomes MF (1989). Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. *Heredity* **62**: 411–418.
- Anderson M, Roberts JA (1998). *Arabidopsis*. Sheffield Academic Press: Sheffield, England.
- Aukerman MJ, Hirschfeld M, Wester L, Weaver M, Clack T, Amasino RM *et al.* (1997). A deletion in the *PHYD* gene of the *Arabidopsis* Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. *Plant Cell* **9**: 1317–1326.
- Barton NH, Turelli M (1989). Evolutionary quantitative genetics: how little do we know? *Annu Rev Genet* **23**: 337–370.
- Belsky AJ (1986). Does herbivory benefit plants? A review of the evidence. *Amer Natur* **127**: 870–892.
- Benner BL (1988). Effects of apex removal and nutrient supplementation on branching and seed production in *Thlaspi arvense* (Brassicaceae). *Amer J Bot* **75**: 645–651.
- Bergelson J, Crawley MJ (1992). The effects of grazers on the performance of individuals and populations of scarlet gilia, *Ipomopsis aggregata*. *Oecologia* **90**: 435–444.
- Bergelson J, Juenger T, Crawley MJ (1996). Regrowth following herbivory in *Ipomopsis aggregata*: compensation but not overcompensation. *Amer Natur* **148**: 744–755.
- Buchner A, Faul F, Erdfelder E (1997). G•Power: *a priori*, *post hoc*, and compromise power analyses for the Macintosh (Version 2.1.2) [Computer program] <http://www.psych.uni-duesseldorf.de/aap/projects/gpower/>. University of Trier: Trier, Germany.
- Cohen J (1992). A power primer. *Psycholog Bull* **112**: 155–159.
- Crews D (2003). Sex determination: where environment and genetics meet. *Evolut Dev* **5**: 1–6.
- Dillon WR, Goldstein M (1984). *Multivariate Analysis. Methods and Applications*. John Wiley & Sons: New York.
- Donohue K (2002). Germination timing influences natural selection on life-history characters in *Arabidopsis thaliana*. *Ecology* **83**: 1006–1016.
- Dorn LA, Pyle EH, Schmitt J (2000). Plasticity to cues and resources in *Arabidopsis thaliana*: testing for adaptive values and costs. *Evolution* **54**: 1982–1994.
- Hay A, Craft J, Tsiantis M (2004). Plant hormones and homeoboxes: bridging the gap? *BioEssays* **26**: 395–404.
- Hedden P, Phillips AL (2000). Gibberellin metabolism: new insights revealed by the genes. *Trends Plant Sci* **5**: 523–530.
- Huhta A-P, Hellstrom K, Rautio P, Tuomi J (2000a). A test of the compensatory continuum: fertilization increases and below-ground competition decreases the grazing tolerance of tall wormseed mustard (*Erysimum strictum*). *Evol Ecol* **14**: 353–372.
- Huhta A-P, Lennartsson T, Tuomi J, Rautio P, Laine K (2000b). Tolerance of *Gentianella campestris* in relation to damage intensity: an interplay between apical dominance and herbivory. *Evol Ecol* **14**: 373–392.
- Jackson RB, Linder CR, Lynch M, Purugganan M, Somerville S, Thayer SS (2002). Linking molecular insight and ecological research. *Trends Ecol Evol* **17**: 409–414.
- JMPIN (2001). *JMPIN Version 4.0.4*. SAS Institute Inc.: Cary, NC.
- Juenger T, Lennartsson T, Tuomi J (2000). The evolution of tolerance to damage in *Gentianella campestris*: natural selection and the quantitative genetics of tolerance. *Evol Ecol* **14**: 393–419.
- Kamiya Y, Garcia-Martinez JL (1999). Regulation of gibberellin biosynthesis by light. *Curr Opin Plant Biol* **2**: 398–403.
- Koornneef M, Elgersma A, Hanhart CJ, van Loenen-Martinet EP, van Rijn L, Zeevaart JAD (1985). A gibberellin insensitive mutant of *Arabidopsis thaliana*. *Physiol Plantarum* **65**: 33–39.
- Koornneef M, van der Veen JH (1980). Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *Theoret Appl Genet* **58**: 257–263.

- Lennartsson T, Tuomi J, Nilsson P (1997). Evidence for an evolutionary history of overcompensation in the grassland biennial *Gentianella campestris* (Gentianaceae). *Am Natur* **149**: 1147–1155.
- Maschinski J, Whitham TG (1989). The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *Am Natur* **134**: 1–19.
- Matos M, Rose MR, Pité MTR, Rego C, Avelar T (2000). Adaptation to the laboratory environment in *Drosophila subobscura*. *J Evol Biol* **13**: 9–19.
- Mopper S, Maschinski J, Cobb N, Whitham TG (1991). A new look at habitat structure: consequences of herbivore-modified plant architecture. In: Bell SS, McCoy ED, Mushinsky HR (eds) *Habitat Structure: the Physical Arrangement of Objects in Space*. Chapman and Hall: New York, pp 260–280.
- Moran MD (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *OIKOS* **100**: 403–405.
- Napp-Zinn K (1985). *Arabidopsis thaliana*. In: Halevy AH (ed) *CRC Handbook of Flowering*. CRC: Boca Raton, FL, pp 492–503.
- Paige KN (1994). Herbivory and *Ipomopsis aggregata*: differences in response, differences in experimental protocol: a reply to Bergelson and Crawley. *Am Nat* **143**: 739–749.
- Paige KN (1999). Regrowth following ungulate herbivory in *Ipomopsis aggregata*: geographic evidence for overcompensation. *Oecologia* **118**: 316–323.
- Paige KN, Whitham TG (1987). Overcompensation in response to mammalian herbivory: the advantage of being eaten. *Am Nat* **129**: 407–416.
- Pang PP, Meyerowitz EM (1987). *Arabidopsis thaliana*: a model system for plant molecular biology. *Bio/Technology* **5**: 1177–1181.
- Phillips AL, Ward DA, Uknes S, Appleford NEJ, Lange T, Huttly AK *et al* (1995). Isolation and expression of three gibberellin 20-oxidase cDNA clones from *Arabidopsis*. *Plant Physiol* **108**: 1049–1057.
- Pigliucci M (1996). How organisms respond to environmental changes: from phenotypes to molecules. *Trends Ecol Evol* **11**: 168–173.
- Pigliucci M (2001). *Phenotypic Plasticity: Beyond Nature Versus Nurture*. Johns Hopkins University Press: Baltimore.
- Pigliucci M, Marlow ET (2001). Differentiation for flowering time and phenotypic integration in *Arabidopsis thaliana* in response to season length and vernalization. *Oecologia* **127**: 501–508.
- Pigliucci M, Schlichting CD (1995). Reaction norms of *Arabidopsis* (Brassicaceae) III. Response to nutrients in 26 populations from a worldwide collection. *Am J Bot* **82**: 1117–1125.
- Pigliucci M, Schlichting CD (1997). On the limits of quantitative genetics for the study of phenotypic evolution. *Acta Biotheoret* **45**: 143–160.
- Pigliucci M, Schmitt J (2004). Phenotypic plasticity to foliar and neutral shade in gibberellin mutants of *Arabidopsis thaliana*. *Ecol Ecol Res* **6**: 243–259.
- Purugganan MD, Suddith JI (1998). Molecular population genetics of the *Arabidopsis* CAULIFLOWER regulatory gene: non neutral evolution and naturally occurring variation in floral homeotic function. *Proc Natl Acad Sci USA* **95**: 8130–8134.
- Rosenthal JP, Kotanen PM (1994). Terrestrial plant tolerance to herbivory. *Trends Ecol Evol* **9**: 145–148.
- Ross JJ (1994). Recent advances in the study of gibberellin mutants. *Plant Growth Regul* **15**: 193–206.
- Ross JJ, Murfet IC, Reid JB (1997). Gibberellin mutants. *Physiol Plantarum* **100**: 550–560.
- Scheiner SM (1993). Genetics and evolution of phenotypic plasticity. *Annu Rev Ecol Systemat* **24**: 35–68.
- Schlichting CD, Smith H (2002). Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. *Evolut Ecol* **16**: 189–211.
- Schmitt J, McCormac AC, Smith H (1995). A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *Am Nat* **146**: 937–953.
- Sgrò CM, Partridge L (2000). Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *Am Nat* **156**: 341–353.
- Sokal RR, Rohlf FJ (1981). *Biometry: the Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company: New York.
- Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J (1999). Dynamics of disease resistance polymorphism at the *RPM1* locus of *Arabidopsis*. *Nature* **400**: 667–671.
- Tiffin P (2000). Are tolerance, avoidance, and antibiosis evolutionary and ecologically equivalent responses of plants to herbivory? *Am Nat* **155**: 128–138.
- Van Der Meijden E (1990). Herbivory as a trigger for growth. *Funct Ecol* **4**: 597–598.
- Weinig C, Stinchcombe JR, Schmitt J (2003). Evolutionary genetics of resistance and tolerance to natural herbivory in *Arabidopsis thaliana*. *Evolution* **57**: 1270–1280.
- Westerman JM (1970). Genotype-environment interaction and developmental regulation in *Arabidopsis thaliana*. IV. Wild material; analysis. *Heredity* **26**: 383–395.
- Zeevaart JAD, Talon M (1992). *Gibberellin mutants in Arabidopsis thaliana*. In: Karssen CM, van Loon LC, Vreugdenhil D (eds) *Progress in Plant Growth Regulation*. Kluwer Academic Publishers: the Netherlands.
- Zhang J, Lechowicz MJ (1994). Correlation between time of flowering and phenotypic plasticity in *Arabidopsis thaliana* (Brassicaceae). *Am J Bot* **81**: 1336–1342.

Supplementary Information accompanies the paper on Heredity website (<http://www.nature.com/hdy>)