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Phenotypic plasticity in response to foliar and neutral shade in gibberellin mutants of *Arabidopsis thaliana*

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ABSTRACT

To examine the role of gibberellins in regulating plasticity to foliage shade, we characterized the reaction norms of gibberellin-insensitive and -deficient mutants of *Arabidopsis thaliana* to variation in photosynthetically active radiation and in the ratio of red : far red light (R : FR). We asked: (1) Do mutations affecting the gibberellin signalling system alter the phenotypic plasticity of *A. thaliana* to foliage shade? (2) Do gibberellin-deficient and gibberellin-insensitive mutants have different reaction norms? (3) If a mutation in gibberellin signalling alters reaction norms to foliage shade, does it affect resource-mediated plasticity to reduced photosynthetically active radiation, or phytochrome-mediated plasticity to the R : FR cue? Mutations at gibberellin signalling loci altered the reaction norms of *A. thaliana*, but had greater impact on trait means than on plasticity. There were clear quantitative differences in reaction norms among gibberellin loci, but the gibberellin-insensitive mutant *gai-1* was not phenotypically distinguishable from the gibberellin-deficient *ga1-5*, *ga1-6*, *ga4-1* and *ga5-1*. The major effect of mutations on the shape of reaction norms was detected for fruit production: some of the gibberellin mutants that we examined dramatically *increased* reproductive fitness relative to the wild type under the favourable conditions and unlimited growing season encountered in the greenhouse. While this fitness advantage might not occur under the stricter selective regime imposed by field conditions, it does demonstrate that mutations at major regulatory loci can significantly positively affect fitness, depending on the environmental circumstances.

Keywords: gibberellin, irradiance, mutants, phenotypic plasticity, red : far red light ratio, shade avoidance.

INTRODUCTION

The study of phenotypic plasticity has historically focused on ecological and evolutionary issues (Bradshaw, 1965; Pigliucci, 2001). Comparatively little attention has been devoted to the genetics (Scheiner, 1993) and especially the mechanistic basis of plasticity (Jackson *et al.*, 2002). This situation has started to change in recent years thanks to ecological investigations of the reaction norms of mutants affecting the response to environmental

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heterogeneity in a few model systems (Schmitt *et al.*, 1995; van Tienderen *et al.*, 1996; Callahan *et al.*, 1999). At least some of these loci are candidate genes for what have been termed 'plasticity genes' (Schlichting and Pigliucci, 1995) – that is, genetic elements coding for proteins that specifically sense and initiate the response to environmental changes (although, of course, downstream transducing and effecting elements are also part of the genetic basis of a plastic response).

One of the better studied types of phenotypic plasticity from this new organismal-molecular perspective is the reaction of plants to foliage shade (Givnish, 1982; Casal and Smith, 1989; Schmitt *et al.*, 1999). Much of this research has focused on the so-called 'shade avoidance' response – that is, the ability of plants to react to the reduced ratio of red to far red wavelengths (R : FR) transmitted or reflected from green vegetation as a cue to present or future competition. Photoreceptors from the phytochrome gene family perceive this shift in light quality and initiate a cascade of developmental processes that dramatically alters the morphology of the plant (Smith, 1982). These changes include earlier flowering, increased apical dominance and decreased branching. However, in natural environments, the R : FR ratio often varies simultaneously with the amount of photosynthetically active radiation. Plasticity to foliage shade, therefore, involves both phytochrome-mediated responses to the R : FR cue and response to low irradiance (which may involve both active developmental plasticity and passive growth responses to light availability).

If the phytochromes perceive light and initiate a cascade of developmental phenomena, how are these signals transduced and effected? Compared with our understanding of phytochrome action, much less is known about what happens after the reception of light signals, but hormones must be an integral part of the picture. Gibberellins in particular have been implicated in several types of responses to light, including photoperiod (Fink *et al.*, 1997; Junttila *et al.*, 1997) and the R : FR ratio (Dahanayake and Galwey, 1999; Olsen and Junttila, 2002). Chory and Li (1997) have reviewed the complex relationship between gibberellins and phytochromes based on the available experimental evidence. The general elongated phenotype and early flowering of *phyB* mutants resembles the phenotype induced by excess gibberellin, either by exogenous application or because of constitutive expression induced by mutation. Evidence for a direct effect of light on gibberellin comes from studies of *phyB*-deficient mutants such as *ein* in *Brassica* and $ma3^{R}$ in sorghum, which are characterized by elevated concentrations of gibberellin (Foster and Morgan, 1995; Devlin *et al.*, 1997). However, such increases in gibberellin accumulation are environment-dependent; furthermore, *phyB* mutations in *Arabidopsis* and other species cause an increase in responsiveness to gibberellin while not altering the accumulation of the hormone *in vivo* (Chory and Li, 1997). These and more recent findings suggest that the phytochrome–gibberellin relationship is not a simple one (e.g. Cowling and Harberd, 1999; Potter *et al.*, 1999; van Tuinen *et al.*, 1999), and may involve distinct signalling pathways that interact under specific environmental conditions.

In this study, we examined the effects of mutations affecting the production of, or sensitivity to, gibberellin hormones on phenotypic plasticity to vegetation shade in the annual weed *Arabidopsis thaliana*. We studied the reaction to light of five mutants and the wild type background from which they were isolated. Four of these mutants are deficient in biosynthetic enzymes at different steps in the production of gibberellins (Koornneef and Veen, 1980; Kamiya and García-Martínez, 1999). The remaining mutant is gibberellin-insensitive (or, rather, it has reduced sensitivity to gibberellin), meaning that it produces gibberellin but a key transcription factor along the transduction pathway is non-functional, so that the

signal triggered by the hormone is not transmitted (Koornneef *et al.*, 1985). The mutants and the control line were exposed to normal sunlight and to both neutral shade and a low R : FR ratio simulating foliage shade, with the aim of investigating the mediation by gibberellin of the plant's response to photosynthetic resources (irradiance) and to cues indicating forthcoming competition (light spectral quality in the form of the R : FR ratio). To distinguish between these two possibilities, the two shade treatments were therefore characterized by the same *amount* of incident light, but by different *spectral compositions*.

We focused on the following questions: (1) Do mutations in gibberellin signalling alter the phenotypic plasticity of *A. thaliana* to foliage shade and, if so, which morphological and life-history traits are affected? (2) Are the reaction norms of gibberellin-deficient mutants different from those of the gibberellin-insensitive mutants, and are there measurable differences among the deficient mutants themselves (as one could predict from their biochemical characterization; Ross *et al.*, 1997, and see below)? (3) If a mutation in the gibberellin pathways affects plasticity to foliage shade, does it alter the response to lower photosynthetically active radiation *per se* (i.e. regardless of spectral composition), or does it act through R : FR-mediated phytochrome pathways? The first outcome would suggest that gibberellin signalling acts rather independently from the shade-avoidance phytochromes, and perhaps in concert with irradiance photoreceptors such as cryptochromes (Lasceve *et al.*, 1999; Lin, 2000); in the second case, gibberellin pathways might interact with the phytochromes as part of the shade-avoidance syndrome.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana (Brassicaceae) is a widely naturalized weedy annual plant. The life cycle comprises several stages defined by major transitions in the morphology of the plant and by accompanying changes in the patterns of gene expression (Hempel and Feldman, 1994). The results of our experiment are presented roughly following the progression from seedling to vegetative to reproductive and senescing stages, focusing in particular on the contrast between the vegetative and reproductive phases.

The *Arabidopsis* Information Resource Center provided the genetically homogeneous lines used for this work (references at www.arabidopsis.org). Landsberg *erecta* (CS–20) is the genetic background from which the mutants were isolated. This 'wild type' is actually a mutant, *erecta*, originally derived from a German natural population. This mutation causes the production of an erect, as opposed to a more prostrate, stem. While this can obviously influence the effectiveness of phenotypic plasticity in response to light, *erecta* is still the appropriate control for our experiment, since all mutants were originated from that genetic background. Four of the five mutants examined in this study are gibberellin-deficient – that is, the mutation precludes the formation and accumulation of gibberellin in the plant. Consequently, under normal conditions, all these mutants are dwarf and some show reduced germination rate. Two mutants, *ga1-5* and *ga1-6*, are allelic at the same locus, but *ga1-6* has more severe phenotypic effects. Both these mutants are 'leaky' – that is, they still produce gibberellin in measurable quantity (www.arabidopsis.org). Importantly, these mutations block the gibberellin pathway very early (Ross *et al.*, 1997). The remaining two gibberellin-deficient lines that we studied (*ga4-1* and *ga5-1*) show a similar general phenotype, but each maps to a distinct genetic locus, and both are recessive 'leaky' alleles

(Zeevaart and Talon, 1992). *ga5* is blocked at an intermediate step in the pathway, and *ga4* is interrupted at a still later step compared with *ga1*. In contrast to all of these, *gai-1* is a gibberellin-insensitive line (it actually has a low level of sensitivity to externally applied gibberellin): bioactive gibberellin is produced and accumulates in the plant's tissues, but it is ineffective, possibly because the mutation damaged a key transcription factor in the transduction pathway (Ross, 1994). *gai-1* is insensitive to externally applied gibberellin, unlike all the other mutants used here. Not surprisingly, phenotypically this mutant resembles gibberellin-deficient genotypes, although the genetic and physiological bases of the defect are entirely different.

We exposed the five mutant lines and the Landsberg *erecta* inbred line to three experimental greenhouse treatments:

1. *Control*, with sunlight reaching the plants through a transparent vinyl screen (R : FR ratio = 1.12; light intensity at noon on a clear day, approximately 325 $\mu M \cdot m^{-2} \cdot s^{-1}$).
2. *Neutral shade*, with a neutral shade cloth layered on the vinyl, reducing photosynthetically active radiation to approximately 50 $\mu M \cdot m^{-2} \cdot s^{-1}$ and the R : FR ratio to around 1.03 (similar to the control).
3. *Simulated foliage* (low R : FR ratio), in which plants were shaded with clear vinyl painted with a dye (chemical composition and preparation described in Lee, 1985), which reduces irradiance to approximately 60 $\mu M \cdot m^{-2} \cdot s^{-1}$ (similar to the neutral shade level) and the R : FR ratio to 0.63 (much lower than both control and neutral shade treatments).

This set-up allowed us to partition plastic responses to foliage shade into resource-mediated responses to photosynthetically active radiation (neutral shade *vs* control treatments), and phytochrome-mediated responses to the R : FR cue (low R : FR shade *vs* neutral shade treatments).

Plants in individual plastic pine cells 2.5 cm in diameter and 15.2 cm deep were arranged in racks. The racks were then laid out on benches in the Brown University greenhouse (latitude 41° 49.5′), surrounded by metal grids supporting the screens. Aluminium foil was placed on the sides of the grids to increase internal reflectivity and minimize edge effects. The foil did not extend for the full height of the metal grids to ensure air circulation. The treatments were replicated in three blocks, with each block containing five randomly distributed replicates of each line/treatment combination, for an experiment-wise total of 15 replicates per line/treatment combination. The trays were randomly rotated within blocks at regular intervals (twice a week for the first month, once a week thereafter) to reduce micro-environmental effects.

Seeds imbibed with water were incubated for a week in the dark at 4°C to stimulate and synchronize germination, and planted in late June. Plants thus experienced the naturally occurring long-day photoperiod: 15 h 1 min at the beginning of the experiment, 11 h 49 min towards the end (it is relevant to note that gibberellin promotes flowering more strongly under short than under long days; Blázquez *et al.*, 1998). The soil was Metro Mix 350, containing processed bark to improve aeration and wettability. Germination occurred 5 days after planting. The experiment was continued until senescence of all reproductively active individuals, at the end of the summer. To minimize mechanical interference, plants were regularly bottom-watered after seedling establishment, and were fertilized with Peters Professional Fertilizers Water Soluble (20–10–20 N–P–K), given at a rate of 50 ppm every

other day (Peat-Lite Formula). Plants were harvested individually, approximately 1 week after the first siliques had opened on each individual (to allow time for seed ripening).

The following characters were scored:

1. Hypocotyl length, a seedling trait.
2. Bolting time, which marks the switch to the reproductive phase and distinguishes ecologically different populations under natural conditions (Westerman, 1970; Jones, 1971).
3. Number of leaves at bolting, an index of the developmental stage at which the reproductive phase is initiated.
4. Height at flowering – that is, the height of the first flower on the main stem when it opens, an architectural character.
5. Time from bolting to senescence, an estimate of the duration of the reproductive phase of the life cycle.
6. Final height, the length of the main reproductive stem at senescence.
7. Number of basal branches, a measure of plant architecture.
8. Number of lateral branches on the main shoot axis (usually correlated with reproductive fitness; Pigliucci and Schlichting, 1996).
9. Total number of fruits produced (by all inflorescences), an estimate of lifetime reproductive fitness in this annual species (since seed number is highly correlated with fruit number; Westerman, 1970).

Statistical analyses

Statistical analyses were performed using Procedure GLM in SYSTAT (2000). Data were checked for normality and heteroscedasticity (Sokal and Rohlf, 1995); it was necessary to log-transform only fruit production. Analyses of variance were carried out to test for effects of: line (genetic variation among mutants/wild type), treatment (overall plasticity), line × treatment interaction (genetic differences in plasticity among lines) and block (residual micro-environmental effects). We considered all effects fixed (since we were interested in the differences among these specific lines and treatments) and therefore tested them over the error term. Because of the multiple tests from the analyses of variance (nine traits), *P*-values were evaluated against adjusted alpha-values obtained by a sequential Bonferroni correction (Rice, 1989). However, since such corrections tend to be overly statistically conservative, we were sensitive to interesting cases of biological effects that did not reach statistical significance but may deserve further study (as strongly advocated by Moran, 2003).

To test more specific hypotheses about the differences among genotypes, derived from the available biochemical characterization of the mutants, we also ran three kinds of *a priori* contrasts for the traits that yielded a significant genotype × environment interaction in the analysis of variance: (a) between the wild type and all mutants, (b) between the sensitive mutants and the deficient mutant and (c) between the two alleles at the *GA1* locus. The contrasts compared the plasticities of the lines in response to photosynthetically active radiation (PAR; high light *vs* neutral shade treatments), PAR + R : FR (high light *vs* simulated foliar shade treatments) and R : FR (low neutral *vs* simulated foliar shade light). These are, therefore, contrasts of genotype–environment interaction terms, which were made possible by the flexible 'hypothesis testing' routine in GLM of SYSTAT and by its

'specify' command. The set of contrasts we chose is not orthogonal, which means that the nominal alpha-value was not matched and that the results of these tests are to be considered indicative (but, again, see Moran, 2003). We did not run specific contrasts to test the possibility of differences among the various sensitive mutants because of the high number of statistical tests that would have resulted. However, we did visually inspect these mutants' reaction norms and the degree of overlap among their confidence intervals. Type III mean squares were used for all analyses, as usual in these cases, to account for the occasional loss of plants in some cells (no cells were empty). Data are presented as reaction norms of genotype × treatment means (untransformed for ease of comparison), with ellipses grouping together within-environment means that did not differ significantly from each other after a one-way analysis of variance.

RESULTS

We found a highly significant overall difference among lines across treatments for all traits examined (Table 1, Figs 1–3), with the mutants usually being characterized by shorter hypocotyls, delayed bolting time, a larger number of leaves, a shorter height at flowering, a longer reproductive period, a shorter final height, more basal and lateral branches and more fruits. There was also a significant effect of treatment on all characters examined, with a tendency for the neutral shade to have the same effect as the low R : FR environment,

Table 1. Results of analysis of variance for the effects of line, treatments, micro-environmental effects and genetic variation for plasticity in six wild type and mutant lines

Trait	Line (d.f. = 5)	Treatment (d.f. = 2)	Block (d.f. = 2)	Treatment × line (d.f. = 10)	Error (d.f. = 142–206)
Hypocotyl length	**3.88 (<0.0001)**	**3.54 (<0.0001)**	**0.37 (0.0042)**	0.06 (0.5747)	0.07
Time to bolting	**225.55 (< 0.0001)**	**1278.84 (<0.0001)**	**107.81 (<0.0001)**	12.02 (0.1353)	7.90
Number of leaves	**1.08 (<0.0001)**	**2.65 (<0.0001)**	**0.13 (0.0055)**	0.04 (0.0524)	0.02
Height at flowering	**1361.37 (<0.0001)**	**1628.32 (0.0001)**	16.33 (0.6875)	**124.68 (0.0027)**	43.45
Duration of reproduction	**3800.00 (<0.0001)**	**2481.26 (<0.0001)**	**1490.17 (0.0002)**	81.97 (0.8944)	167.31
Final height	**4995.94 (0.0004)**	**64694.80 (<0.0001)**	3726.19 (0.0284)	1164.57 (0.3363)	1021.04
Basal branches	**27.93 (<0.0001)**	**69.47 (<0.0001)**	**15.69 (0.0093)**	**12.12 (0.0002)**	3.24
Lateral branches	**20.30 (<0.0001)**	**27.07 (<0.0001)**	1.55 (0.3654)	1.99 (0.2352)	1.53
Fruit production (log-transformed)	**18.07 (<0.0001)**	**26.25 (<0.0001)**	0.34 (0.7010)	**2.42 (0.0081)**	0.95

Note: Mean squares are reported, with *P*-values in parentheses. **Boldface** indicates significance following a sequential Bonferroni correction.

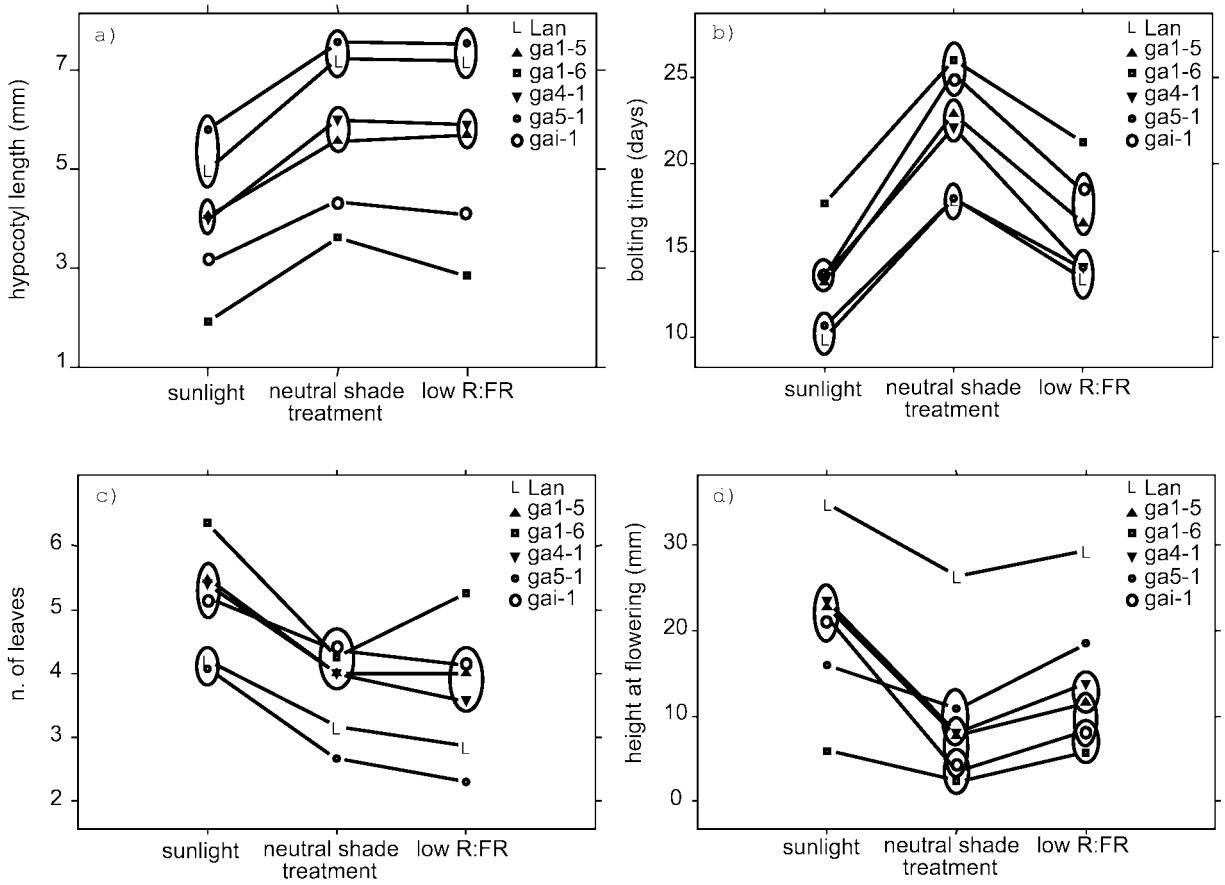

Fig. 1. Reaction norms to normal light (control), neutral shade (shade) and reduced red to far red ratio (low R : FR) for four gibberellin-deficient mutants, one gibberellin-insensitive mutant (cs63) and the wild type genetic background from which they were isolated (Landsberg). Seedling, vegetative and early reproductive traits: (a) hypocotyl length; (b) time to bolting; (c) number of basal leaves; (d) height at flowering. Ellipses indicate within-treatment means that are significantly different from each other.

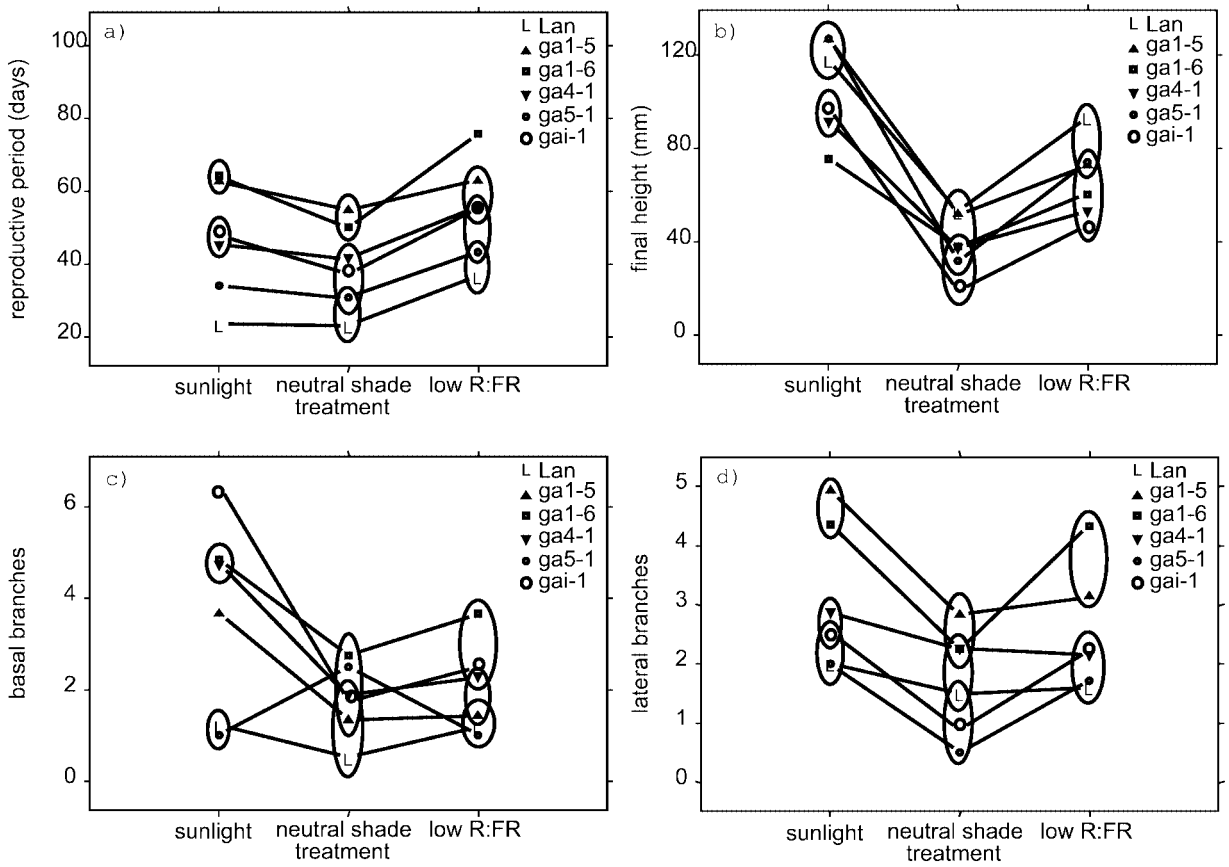

Fig. 2. Reaction norms to normal light (control), neutral shade (shade) and reduced red to far red ratio (low R : FR) for four gibberellin-deficient mutants, one gibberellin-insensitive mutant (cs63) and the wild type genetic background from which they were isolated (Landsberg). Late reproductive traits: (a) time to senescence (from bolting); (b) final height (of the main stem); (c) number of basal inflorescences; (d) number of lateral inflorescences (on the main stem). Ellipses indicate within-treatment means that are significantly different from each other.

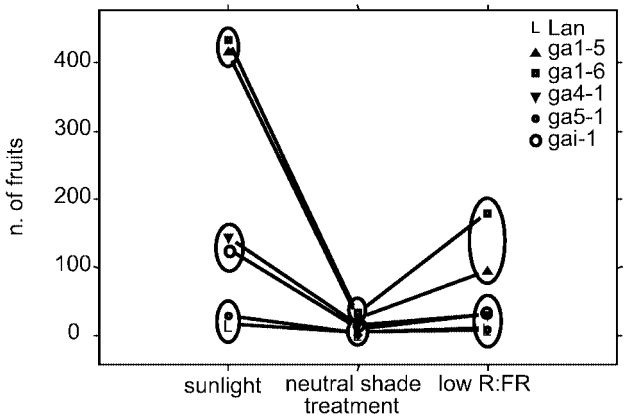

Fig. 3. Reproductive fitness (total fruit production) reaction norms to normal light (control), neutral shade (shade) and reduced red to far red ratio (low R : FR) for four gibberellin-deficient mutants, one gibberellin-insensitive mutant (cs63) and the wild type genetic background from which they were isolated (Landsberg). Ellipses indicate within-treatment means that are significantly different from each other.

especially on reproductive output (Table 1, Figs 1–3). Only three traits displayed a significant overall genotype × environment interaction: height at flowering, number of basal branches and fruit production, with a marginally non-significant effect for leaf number.

Response to PAR + R : FR (full light *vs* simulated foliar shade treatments)

This comparison focuses on the joint effect of light quantity and quality, since both variables changed between the full light and the simulated foliar shade treatments, as would happen under natural conditions. We found marginally significant differences in the plasticity of fruit production between the wild type and all mutants, in height at flowering and basal branching between the insensitive mutants and the deficient mutant, and in height at flowering between the two forms of *GA1*. (Table 2).

Landsberg *erecta* was essentially unresponsive to simulated foliar shade, always yielding few fruits, while some mutants, especially *ga1-5* and *ga1-6*, produced many more fruits under sunlight than under low R : FR shade (Fig. 3). The difference between the insensitive mutants and the deficient one for height at flowering was probably due to the less plastic reaction norms of *ga5-1* and *ga1-6* (Fig. 1d). The insensitive mutant was more plastic in the response of basal branching to foliar shade than some of the deficient ones, especially *ga5-1* (Fig. 2c). Lastly, the different plasticity for height at flowering of the two allelic *ga1* mutants was again due to the low responsiveness of *ga1-6* (Fig. 1d).

Response to PAR (full light *vs* neutral shade treatments)

When comparing the full sunlight with the neutral shade treatments, we were assessing the plants' responses to changes in total photosynthetically active radiation, while the spectral quality of the incident light was similar. Significant interaction contrasts indicated no differences in the plasticity of fruit production to photosynthetically active radiation

Table 2. *A priori* contrasts examining differences in plasticity to PAR + R : FR (full light *vs* simulated foliar shade treatments) among specific sets of lines for the traits with a significant overall genotype × environment interaction (see Table 1)

Trait	Hypothesis mean square	Error mean square	*F*-ratio	*P*-value
Wild type *vs* all mutants				
Height at flowering	0.28	43.45	0.01	0.9359
Basal branches	7.67	3.24	2.36	0.1264
Fruit production (transformed)	3.50	0.95	3.70	0.0569
Insensitive *vs* deficient mutants				
Height at flowering	416.51	43.45	9.59	**0.0024**
Basal branches	32.03	3.24	9.87	**0.0020**
Fruit production (transformed)	0.35	0.95	0.37	0.5443
Allelic genes at the *GA1* locus				
Height at flowering	191.42	43.45	4.41	**0.0376**
Basal branches	1.05	3.24	0.32	0.5710
Fruit production (transformed)	0.55	0.95	0.58	0.4485

Note: **Boldface** indicates significance at the $P < 0.05$ criterion.

between the wild type and all mutants (Table 3), while there were significant differences in plasticity of height at flowering and basal branching in the insensitive versus deficient gibberellin mutants, and in plasticity of height at flowering and fruit production between the plants carrying the two allelic forms of *GA1* (Table 3).

The lack of statistical difference in the plasticity of fruit production between the wild type and all other mutants was striking given that the reaction norm plots clearly indicated that most mutants (with the exception of *ga5-1*) produced many more fruits than Landsberg *erecta* under full sunlight, while all lines converged towards very little fruit production under neutral shade (Fig. 3). This is a clear example of an incongruence between statistical and biological significance (Dixon and O'Reilly, 1999), and where it would therefore be reasonable to reserve judgement until follow-up studies are carried out.

The different response of the insensitive and deficient mutants in height at flowering was attributable mostly to the fact that *ga5-1* and *ga1-6* were nearly as tall under neutral shade as under sunlight, whereas the remainder of the deficient mutants and the insensitive *gai-1* were significantly taller under sunlight (Fig. 1d). The insensitive mutant produced significantly more basal branches under sunlight than any of the other mutants, while the differences disappeared under neutral shade (Fig. 2c). The difference in plasticity of height at flowering between the two *GA1* alleles was due to the higher plasticity of *ga1-5*, which produced significantly taller plants under normal sunlight.

Response to R : FR (neutral *vs* simulated foliar shade treatments)

The last set of comparisons addressed plasticity to light quality *per se*, by contrasting the low neutral light and the simulated foliar shade treatments. In this case, our planned contrasts detected no significant differences in the plasticity of mutants versus wild type,

Table 3. *A priori* contrasts examining differences in plasticity to photosynthetically active radiation (full light *vs* neutral shade treatments) among specific sets of lines for the traits with a significant overall genotype × environment interaction (see Table 1)

Trait	Hypothesis mean square	Error mean square	*F*-ratio	*P*-value
Wild type *vs* all mutants				
Height at flowering	5.27	43.45	0.12	0.7282
Basal branches	5.08	3.24	1.57	0.2128
Fruit production (transformed)	0.63	0.95	0.66	0.4171
Insensitive *vs* deficient mutants				
Height at flowering	469.90	43.45	10.82	**0.0013**
Basal branches	58.32	3.24	17.97	**<0.0001**
Fruit production (transformed)	0.56	0.95	0.59	0.4446
Allelic genes at the *GA1* locus				
Height at flowering	222.43	43.45	5.12	**0.0252**
Basal branches	0.01	3.24	0.01	0.9455
Fruit production (transformed)	4.00	0.95	4.23	**0.0421**

Note: **Boldface** indicates significance at the $P < 0.05$ criterion.

Table 4. *A priori* contrasts examining differences in plasticity to R : FR (low neutral light *vs* simulated foliar shade treatments) among specific sets of lines for the traits with a significant overall genotype × environment interaction (see Table 1)

Trait	Hypothesis mean square	Error mean square	*F*-ratio	*P*-value
Wild type *vs* all mutants				
Height at flowering	5.25	43.45	0.12	0.7287
Basal branches	0.55	3.24	0.17	0.6816
Fruit production (transformed)	0.55	0.95	0.58	0.4489
Insensitive *vs* deficient mutants				
Height at flowering	1.10	43.45	0.03	0.8738
Basal branches	2.85	3.24	0.88	0.3500
Fruit production (transformed)	1.08	0.95	1.14	0.2875
Allelic genes at the *GA1* locus				
Height at flowering	0.50	43.45	0.01	0.9144
Basal branches	0.57	3.24	0.17	0.6769
Fruit production (transformed)	1.31	0.95	1.38	0.2419

insensitive versus deficient mutants, or allelic mutants for any trait. This indicates that the plants were responding in the same way to alterations in the light spectral quality, although the reaction norms may be suggestive of some subtle differences that our experimental set-up did not have the power to demonstrate statistically (Table 4).

DISCUSSION

Evolutionary biologists have been calling for a unification of the neo-Darwinian synthesis with developmental and, more recently, molecular biology throughout the last half century (history and references in Schlichting and Pigliucci, 1998; Gould, 2002). If we understand evolutionary theory as more than the study of natural variation, then mutagenesis and molecular developmental biology must provide crucial pieces of the overall puzzle (Callahan *et al.*, 1997). This is all the more so in light of recent studies suggesting that the once common assumption that mutations at high-level regulatory loci are kept in check by natural selection, and therefore do not contribute significantly to evolutionary change, may very well be incorrect (Aukerman *et al.*, 1997; Doebley and Lukens, 1998; Purugganan and Suddith, 1999; Jackson *et al.*, 2002). This study was intended as a contribution towards understanding how molecular and organismal biology can be considered simultaneously to yield insights into the evolution of complex phenotypes, such as responses of plants to light intensity and spectral quality. Obviously, comprehensive answers are not going to come from any individual study, but we think the approach taken here is a significant component of the solution of the overall puzzle.

Effects of deficiency of, and insensitivity to, gibberellin on reaction norms to light availability

Our results indicate that gibberellin deficiency and insensitivity can alter reaction norms to light availability in *A. thaliana*. However, the effects of mutations on trait means and plasticities depended on the specific line and upon the light environment. In no case were the lines we studied significantly heterogeneous in their plasticities for seedling and vegetative characters. Gibberellin-insensitive or -deficient plants had shorter hypocotyls, flowered later, produced more leaves and had longer reproductive periods than the wild type, but did so in a similar fashion regardless of the environment. This phenotypic syndrome is in agreement with what we know of the action of gibberellin in stimulating stem elongation (Lopez-Juez *et al.*, 1995; Grindal *et al.*, 1998; Dahanayake and Galwey, 1999) and flowering (Bagnall, 1992; Chory and Li, 1997; Fink *et al.*, 1997).

Mutations affecting gibberellin did significantly alter the phenotypic plasticity of *A. thaliana*, but only later in the life cycle, and specifically for height at flowering, number of basal branches and reproductive fitness. For these traits we have to distinguish between effects of the mutations on the height of the reaction norm (i.e. the across-environment mean) and its shape (i.e. the pattern and amount of plasticity). The effects of the mutations on the across-environment means of reproductive characters were again in line with what we know of the action of gibberellin-like compounds (Chory and Li, 1997; Ross *et al.*, 1997). Namely, gibberellin deficiency or insensitivity caused a dwarf or semi-dwarf phenotype as well as increased branching.

The effects on the plasticity of reproductive traits when compared with the wild type control were varied and depended on the particular mutant. In general, lack of gibberellin (or responsiveness to it) did not significantly alter the normally low level of plasticity displayed by Landsberg *erecta* to either irradiance (PAR) or light quality (R : FR), except for fruit production. This is very different from what has been observed for mutations affecting some of the phytochrome receptors (Pigliucci and Schmitt, 1999), where the

mutants lose plasticity to light quality when compared with the wild type. However, we did observe some potentially biologically significant heterogeneity of reaction norms among deficiency mutants, suggesting that disruption of gibberellin pathways at different steps may have different effects on plasticity to shade. This heterogeneity among mutants may have been a result of the fact that these mutations do not completely prevent biosynthesis of gibberellins (because of the partial redundancy of the relevant pathways: Ross *et al.*, 1997) and differ quantitatively from each other (due to different degrees of 'leakiness' of the different mutations: Zeevaart and Talon, 1992). Therefore, the mutant genotypes are clearly still able to alter the level and action of bioactive, gibberellin-related compounds in response to environmental cues. Our results also indicate that gibberellin loci markedly affect trait means, at least in early flowering populations of *A. thaliana* such as Landsberg. It would be interesting to determine the effects of mutations at the same loci in lines of *A. thaliana*, which, unlike Landsberg and its derivative *erecta*, are naturally more plastic and late flowering.

Several molecular studies have implied a role for gibberellin in response to foliage shade largely independent from phytochromes (Lopez-Juez *et al.*, 1995; Chory and Li, 1997; but see van Tuinen *et al.*, 1999), and our results appear to show that this role may be fairly limited. However, it is interesting to note that gibberellins are involved in other responses to light, such as to photoperiod (Fink *et al.*, 1997), implying that the photoreceptor–hormone relationship in response to light depends on the particular aspect of light availability being considered.

Differences between and within gibberellin-insensitive and -deficient mutants

The two leaky alleles at the same locus (*ga1-5* and *ga1-6*) had reaction norms of different height (i.e. different average response) for some traits, most notably hypocotyl length (*ga1-5* was taller), number of leaves (*ga1-6* produced more), bolting time (*ga1-6* flowered later), height at flowering and final height (*ga1-5* was taller in both cases), as well as basal branches (more in *ga1-6*). However, these two mutants differed in their plasticities only for height at flowering, with the dissimilarity being manifest in their responses to levels of photosynthetically active radiation, not the R : FR cue. As mentioned above, these differences are likely to be attributable to quantitative differences in the leakiness of the two alleles.

Although we did not set out specific contrasts to analyse differences among the various deficient mutants (since we had no definite *a priori* hypotheses to guide such contrasts, and because that would have unnecessarily multiplied the number of simultaneous statistical tests), the reaction norm plots and associated confidence intervals clearly showed some degree of heterogeneity. This suggests that halting the gibberellin pathway at different points has distinct effects on the reaction norm, although the severity of the particular mutation may again have played a role. For example, *ga4-1* and especially *ga5-1* showed reaction norms very similar to the wild type for several traits; these two mutations affect the gibberellin pathway later than the other ones, and it is possible that the resulting biological activity is not too different from that of the wild type.

The gibberellin-insensitive mutant, *gai-1*, behaved in a fashion indistinguishable from the bulk of gibberellin-deficient lines for all traits except height at flowering and basal branches in the PAR + R : FR (Table 2) and PAR (Table 3) contrasts. This largely, but not entirely, confirms that its phenotype is similar to that of the gibberellin-deficient mutants,

despite the qualitative biochemical differences between the two types of mutation (Ross *et al.*, 1997). Recently, evolutionary biologists have seriously considered the implications of regulatory genes with biochemically distinct but phenotypically redundant functions (Pickett and Meeks-Wagner, 1995; Wagner, 1999). Specifically, genetic redundancy to some extent decouples phenotypic from genetic variation, in that different mechanistic pathways can lead to similar phenotypes. Barring additional pleiotropic effects, natural selection will favour some phenotypes regardless of the underlying genetic architecture that produces them, thereby generating redundancy.

Gibberellin mutations and reproductive fitness

An interesting finding in this study is that many of these major regulatory mutations actually *increased* (and none decreased) the reproductive fitness of the plant when compared with the 'wild type' under benign greenhouse conditions (i.e. with an unlimited growing season and ample supply of water and nutrients). This increase in reproductive fitness of the mutant genotypes was environment-specific, being most evident under high light and not at all under neutral shade (i.e. again, under benign conditions). A similar phenomenon has been noted in blue receptor mutants of *A. thaliana*, also only when exposed to high light and given the same unlimited growing season and other resources as in this study (Pigliucci and Schmitt, 1999). That effect disappeared under ecologically more realistic conditions, explaining why blue defective mutants are not common in nature (Callahan *et al.*, 1999). In an experiment with inbred lines from natural populations given an unlimited growing season, selection favoured late flowering plants with many leaves and branches under full sun, but early bolting and fewer leaves and branches under foliage shade (Dorn *et al.*, 2000). These combined findings suggest that if the length of the growing season is extended, Landsberg *erecta* (an early flowering genotype) would actually be better off if the inhibitory effects of gibberellin (and of the cryptochrome: Pigliucci and Schmitt, 1999) on phenotypic plasticity to light availability were reduced. While our and similar results are of limited use in characterizing the ecology of natural genetic variants of this species, they do yield some interesting information on the functional ecology of light receptors and of their transduction pathways.

For example, it is interesting that the increased plasticity of reproductive effort to light shown by some of the lines we studied was most pronounced in the two mutants at the *GA1* locus. These mutants, and to some extent the other two characterized by heightened fruit output (*ga4-1* and *gai-1*), also produced more basal branches than the wild type, particularly under sunlight. The effect of these mutations on plasticity of fruit output can therefore be explained within the context of the particular environment in which the plants were growing. Since they were under benign growing conditions in our experimental set-up, and not at risk of drought or other naturally occurring stresses such as heat or competition, the increase in allocation to reproductive meristems to produce additional inflorescences paid off by translating into augmented reproductive fitness. Consequently, even mutations at major regulatory genes (such as those affecting the action of hormones) can be advantageous under certain conditions. Note that such conditions are not necessarily uncommon for *A. thaliana*, some populations of which live in rather undisturbed and nutrient-rich environments (Napp-Zinn, 1985; Thompson, 1994). In the case of our lines, one needs also to consider that Landsberg has been selected for a fast life cycle, at the cost of lifetime reproductive potential. Mutations that cause a delay in flowering (like most of the

gibberellin ones examined here) will also automatically release this constraint, as has already been observed in this species (Pigliucci *et al.*, 1998).

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