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Comparative studies of reaction norms in *Arabidopsis*. I. Evolution of response to daylength

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ABSTRACT

The evolution of reaction norms (the genotype-specific manifestation of different phenotypes in response to environmental variation) has rarely been addressed from within a phylogenetic comparative framework, despite general agreement that we need a better understanding of how historical and deterministic processes (e.g. selection and constraints) interact to yield a particular pattern of variation of phenotypic plasticity within and across species. In this study, we compare the reaction norms to daylength in eight accessions of *Arabidopsis thaliana* from Scandinavia and three closely related species, *A. arenosa*, *A. lyrata* subsp. *petraea* and *A. suecica*. We find that across-environment means evolved continuously and very rapidly within this group, while plasticity (calculated as the difference between the character value under short photoperiod minus the expression of the same trait under long photoperiod) changed only rarely and especially at the base of the *A. thaliana* clade. Character means co-evolved in a fashion that identifies two functional sets of traits, one during the vegetative phase and the other during the reproductive phase, with trade-offs between characters expressed across the two phases. With a few exceptions, plasticities of different traits tended to evolve largely independently of each other. Several plasticities evolved in concert with their corresponding across-environment means, but by following a pattern of negative correlations that cannot be explained by simple geometrical considerations in environment-phenotype space. Neither the mean nor the plasticity of a focal trait, flowering time, were correlated with differences in phylogenetic relatedness, geographical distance or latitudinal differential. This suggests that neither genetic drift nor adaptation to large-scale geographical factors occurred.

Keywords: *Arabidopsis*, comparative method, daylength, intraspecific phylogeny, reaction norms, phenotypic plasticity.

INTRODUCTION

An organism exhibits phenotypic plasticity if its morphology depends on changes in the external environment (Schmalhausen, 1949). This response to the environment can be adaptive (Sultan, 1987, 1995) and it can occur following one of at least two response modes: anticipatory plasticity or passive reaction to stressful conditions (Schlichting and Pigliucci, 1995). If the plasticity is anticipatory, the organism responds to specific cues from the

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environment that signal a forthcoming change in external conditions, such as shade avoidance in plants induced by an alteration in the ratio between red and far red light (Schmitt *et al.*, 1999), or the response of several animal species to chemicals or other cues associated with predators (e.g. Lively, 1999). If the plasticity is passive, the environmental change is followed by alterations in the phenotype, generally roughly proportional to the change in environmental conditions. For example, the developmental system of *Drosophila* probably reacts to continuous variation in temperature in this manner (e.g. Land *et al.*, 1999).

Although it might be assumed that anticipatory responses are adaptive on functional ecological bases, this is not always the case. For example, Sultan and Bazzaz (1993) exposed *Polygonum persicaria* to a series of environments differing in light quantity. Those genotypes grown under low light produced more leaves than those grown under high light, as expected from a functional ecological standpoint for an adaptive plastic response. However, this resulted in less fruit and stem tissue and, therefore, presumably in lower fitness (measured as a combination of reproductive effort and biomass production). On the other hand, passive responses are not necessarily maladaptive, as suggested by the shifting of reaction norms between tropical and temperate species or populations of *Drosophila* (Morin *et al.*, 1999). Thus, there is no simple relationship among adaptive, non-adaptive, anticipatory and passive plasticity.

Because of these potentially complex correspondences between the type of plasticity and its fitness consequences, adaptive plasticity hypotheses must be tested directly (Schmitt *et al.*, 1995; Dudley and Schmitt, 1996). In particular, we would expect that, if trait plasticities were responding to selection, they may display patterns of directional change across phylogenies (Pigliucci *et al.*, 1999). A test of this hypothesis can be achieved by conducting functional ecological studies in the context of comparative phylogenetics and biogeography. Functional ecological research such as that carried out by Schmitt *et al.* (1995) on shade avoidance in tobacco and *Brassica* addresses whether or not the plasticity is currently adaptive, in the sense that morphological change in response to the environment enhances some component of fitness. The historical approach, by contrast, addresses whether the plasticity evolved as an adaptation, although it may or may not be currently advantageous. To test an adaptive plasticity hypothesis we need an appropriate null hypothesis for the reaction norms to be compared. While for functional studies the best comparison is with genotypes characterized by no or extremely reduced plasticity, for comparative research the most appropriate reaction norms to use as a standard are those that can be reasonably ascribed to taxa ancestral to the species of interest (Doughty, 1995). Several comparative studies of plasticity have been published (e.g. Milbrath *et al.*, 1993; Baskauf and Eickmeier, 1994; Lamade *et al.*, 1994; Windig *et al.*, 1994; Burns, 1995; Saleeba and Guerinot, 1995; Holloway *et al.*, 1997). However, only in a few cases has a phylogenetic hypothesis been incorporated (e.g. Roskam and Brakefield, 1996; Morand *et al.*, 1997; Pigliucci *et al.*, 1999). One of the reasons for this dearth of phylogenetic studies of plasticity is the cumbersome logistics of such experiments, which require extremely large sample sizes.

The type of plasticity studied, of course, determines the range of functional hypotheses and their testability. Therefore, choosing the appropriate environmental cue represents a crucial step in every research programme on phenotypic plasticity. In plants, response to different aspects of light availability is essential for survival and reproduction (Furuya, 1993; Smith, 1995; Schmitt, 1997; Callahan *et al.*, 1999). These include the red : far red ratio, an indicator of vegetation shade and therefore of competition, and daylength, associated

with seasonal changes and determining the total amount of photosynthetically active light available. This paper examines the evolution of the response to daylength within a small clade of the Brassicaceae family. This plasticity has been studied from a physiological and molecular developmental perspective, and we know that it is effected through a variety of photoreceptors (Jakson and Thomas, 1997; Weller *et al.*, 1997), sometimes acting in antagonistic fashion (Mozley and Thomas, 1995). Some of these receptors are also involved in other responses to light availability, such as red : far red ratio, which opens the possibility of co-evolution of related plastic responses, which will be addressed in a forthcoming paper (H. Pollard *et al.*, submitted).

We used plants of the genus *Arabidopsis*, a model system for both molecular developmental biology and ecological genetics (Anderson and Roberts, 1998; Pigliucci, 1998). Much is known about the molecular basis of responses to light in this system (Fuglevand *et al.*, 1996; Whitelam and Devlin, 1997; Somers *et al.*, 1998), but much less is understood about its population biology (e.g. Clauss and Aarssen, 1994; Thompson, 1994; Li *et al.*, 1998; Pigliucci and Schlichting, 1998). We focused on plants collected in Scandinavia, which is part of the natural range for this species and is also characterized by a steep variation in daylength over a relatively short latitudinal range.

Arabidopsis thaliana and its phylogenetic neighbourhood have been subjected to a series of phylogenetic studies (Hylander, 1957; Laibach, 1958; Price *et al.*, 1994; Mummenhoff and Hurka, 1995; O'Kane *et al.*, 1996) to resolve relationships among closely related species. However, *intra*-specific phylogenies are also needed for *micro*-evolutionary studies, such as the one presented here. *A. thaliana* is an excellent candidate for intraspecific comparative studies because of high sequence variation (King *et al.*, 1993; Hardtke *et al.*, 1996) and high level of selfing (Abbott and Gomes, 1989), which reduces the possibility of confounding events of reticulate evolution.

In this paper, we address the following questions: (1) Is it possible to obtain a robust phylogenetic hypothesis for haplotypes of *A. thaliana* collected in a given geographic area? (2) Is there variation among Scandinavian accessions for trait means and plasticities? (3) How did trait means and plasticities evolve along the hypothesized intraspecific phylogeny? (4) Did the means of different traits co-evolve along the phylogeny? (5) Did the plasticities of different traits co-evolve along the phylogeny? (6) Did plasticities and means for the same trait co-evolve, or did they behave as independent characters as originally suggested by Bradshaw (1965)? (7) Is there an association between accession-specific flowering time schedule and phylogenetic, latitudinal or geographic distance? [considering that flowering time is a key component of the life cycle of *Arabidopsis*, which is known to be highly responsive to daylength (Karlsson *et al.*, 1993; Coupland, 1997; Fink *et al.*, 1997)]. The results from these studies provide insights into the evolution of both character means and their associated plasticities and can provide the basis for further research on the deterministic mechanisms (selection and constraints) directing the evolution of reaction norms in natural populations.

MATERIALS AND METHODS

Plant material

Eight Scandinavian accessions of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae), two closely related species [*A. lyrata* (L.) O'Kane & Al-Shehbaz subsp. *petraea* (L.) and

A. arenosa (L.) Lawalrée] and a hybrid species [*A. suecica* (Fries) Norrlin] were used in this experiment. Except for the *A. arenosa* accession, all seeds came from the *Arabidopsis* Information and Management System (AIMS, http://aims.cps.msu.edu/aims/). Of the Scandinavian accessions, two were from Denmark (CS1220 and CS1288), two from Finland (CS1144 and CS1160), two from Norway (CS1436 and CS1643) and two from Sweden (CS1352 and CS1430). The accession number for *A. lyrata* subsp. *petraea* is CS3219 and that for *A. suecica* is CS3220. The *A. arenosa* seeds were collected in Göttingen, Germany and kindly provided by the Botanical Garden there.

Morphological (Hylander, 1957), cpDNA (O'Kane *et al.*, 1996) and rDNA (Mummenhoff and Hurka, 1995) studies support the hypothesis that *A. thaliana* and *A. arenosa* are the putative parents of *A. suecica*. Based on the patterns of nuclear and cpDNA in these taxa, the maternal parent of *A. suecica* is probably *A. thaliana*, while *A. arenosa* is the paternal parent (Mummenhoff and Hurka, 1995). *A. suecica*, an allopolyploid, is found in Sweden, Finland, the Baltic States and Norway (Hylander, 1957; Love, 1961). The two outgroups are distributed mainly throughout the eastern hemisphere: *A. lyrata* subsp. *petraea* can be found in northern, central and eastern Europe, northern Asia and North America; *A. arenosa* is distributed over much of Europe and Siberia (O'Kane and Al-Shehbaz, 1997).

Known latitudes for the chosen *A. thaliana* accessions (excluding Finland 1160 for which the collection site is unknown) vary from 50° to 61°N; known longitudes vary from 6° to 25°E; known altitudes range from 1 to 200 m. At these localities, average daily temperatures vary from about 4°C to 8°C in April and from about 16°C to 18°C in July (Schonwiese and Rapp, 1997). Note that *Arabidopsis* overwinters as a rosette and grows throughout the spring at most locations, even in Scandinavia (Bowman, 1994). Daylength at 50°N is about 13.5–14.5 h in April (approximate time of flowering at that latitude) and 16 h in June; the daylength at 60°N is about 14–15.5 h in April and 18.4 h in June (approximate time of flowering at that latitude).

Handling of plants

Seeds from all 11 accessions were placed in the dark on moist filter paper for 10 days at 4°C. Following vernalization, the seeds were planted in pots of Pro-Mix general-purpose soil (Premier Horticulture Inc.) and were germinated under one of two treatments created using Philips 20 W light bulbs (see below). The trays were bottom-watered as required and fertilized weekly.

Plants were grown in two growth chambers at the University of Tennessee, Knoxville. One chamber was set at 14 h light per day (short day treatment, equivalent to flowering season at 50°N) and the other was set at 22 h light per day (long day treatment, equivalent to flowering season at latitudes further north than our sampling area, to maximize the plant's response). To minimize micro-environmental effects, the trays were switched once per week between chambers and the light treatments were reset until plants began to bolt; afterwards, the rotation was carried out once every 2 weeks. We measured the light intensity and light quality with a portable Li-Cor LI-1800 spectroradiometer, and all trays were placed so that these measurements would be similar for each flat. The light quality for these treatments was equivalent to a 1:1 red:far-red ratio (typical of sunlight), and the light intensity was approximately 55 $\mu M \cdot m^{-2} \cdot s^{-1}$. Temperature was set at 18°C for both treatments.

Various morphological traits were measured to quantify the variation among plants: number of rosette leaves at bolting (meristem allocation to vegetative growth); rosette diameter (extent of vegetative growth); days to bolting (time at which plants switch from the vegetative to the reproductive phase); length of the main stem (a measure of overall size during the reproductive phase); number of basal stems (an indication of plant architecture and potential for late reproduction); time of first seed set (interval between bolting and the ripening of the first seeds); number of fruits on the main stem (a partial measure of reproductive fitness); and number of fruits on the basal stem(s) (partial measure of reproductive fitness). Plasticities were quantified as the trait expression under short photoperiod minus the trait expression under long photoperiod.

Estimating the phylogeny

Relationships among *Arabidopsis* accessions were inferred from chloroplast DNA (cpDNA) sequence variation for three intergenic regions. Leaf material from representative individuals of each accession was snap frozen in liquid nitrogen and stored at −70°C. Total genomic DNA was extracted from the frozen leaves according to methods described by Edwards *et al.* (1991). Isolated DNA was purified by binding it to cellulose, rinsing with low saline buffer over syringe filters, and eluting with high saline buffer (Marechal-Drovard and Guillemaut, 1995; Martin and Cruzan, 1999). Isolation of DNA was confirmed by visualizing bands with ethidium bromide under ultraviolet light after electrophoresis on 1% agarose gels.

We examined genetic variation among accessions by sequencing three intergenic cpDNA regions that have previously been shown to display high variation within and among species [trnT(UGU)-trnF(GAA): Taberlet *et al.*, 1991]. Each intergenic region was amplified using the polymerase chain reaction in 25 μl reactions consisting of approximately 10 ng genomic DNA in 2 mM $MgCl_2$, 1 μM of each primer, 200 μM of each dNTP and 0.5 units of *Taq* DNA polymerase (Promega) in a buffer supplied by the manufacturer. Amplifications were conducted by heating reaction mixtures to 94°C for 1 min, followed by 50–55°C (depending on the primer pair) for 1 min and 72°C for 2 min, for a total of 35 cycles. Amplification products (double-stranded templates) were sequenced in both directions on an ABI Prism Dye Terminator Cycle Sequencing reaction kit on an ABI 373 DNA sequencer (Perkin-Elmer Inc., Foster City, CA). The initial sequence data text files were edited following comparison with the same data displayed in four-colour electropherograms, and any ambiguities or discrepancies between the complementary sequences were scored as missing data. Sequences for all of the accessions were aligned with each other by eye so as to minimize the number of gaps.

The data were analysed using PAUP (versions 3.1.1 and 4.0b1) on PowerPC and Windows 95 operating systems, respectively. For all analyses, *A. arenosa* and *A. petraea* were defined as outgroups. The three intergenic regions were combined and submitted to the program as a single sequence that was 2100 bases long and contained 38 insertion/deletions (in/dels), which were scored separately. Each base position was treated as an unordered character with four possible states and each in/del was treated as an unordered character with two states. Base substitutions were weighted equally, unequally with transitions (ts) down-weighted relative to transversions (tv) by a factor of 2 (ts : tv = 1 : 2; the observed ratio in the data set), or unequally with transitions down-weighted by a factor of 5 (ts : tv = 1 : 5) in separate analyses. Phylogenetic relationships among haplotypes were estimated using

maximum likelihood (excluding in/dels), which is thought to be more appropriate for sequence data than alternative methods (Swofford *et al.*, 1996).

We optimized our maximum likelihood analyses of phylogenetic relationships among accessions by comparing 16 models of sequence evolution that included combinations of four models of nucleotide substitution and four models of among-site rate variation (Swofford *et al.*, 1996; Sullivan *et al.*, 1997; Mason-Gamer *et al.*, 1998). The models were assessed by comparing the likelihood scores estimated for 22 different unrooted trees that were generated from weighted and unweighted parsimony analyses of both the base substitution and in/del data sets using PAUP 4.0b1. The models of sequence evolution that produced the highest scores were compared to other models using likelihood ratio tests (Yang *et al.*, 1994) and were used to estimate phylogenetic relationships among accessions by conducting multiple heuristic searches with the random addition of sequences to examine the likelihood surface for multiple optima.

Strictly speaking, the phylogeny we present here is for maternal haplotypes, not for the populations from which they were derived. This is because there is a low but significant possibility of reticulate evolution within *A. thaliana*, even though this species is characterized by mostly local dispersal and very low to no outcrossing (Abbot and Gomes, 1989).

Statistical analyses

Univariate analysis of variance (ANOVA) provided a means to examine the effect of accession (treated as a fixed effect), treatment (fixed effect), plot (random effect nested within treatment) and accession × treatment interactions. Twelve replicates of each family were placed in each of the two daylength treatments. There were six trays per treatment (three on the top shelf and three on the bottom shelf of each growth chamber), with two randomly placed seedlings per accession per tray. Due to some mortality, trays were placed into groups of three; this grouping was referred to as 'plot'. These plots were nested within the treatments/chambers. The resulting experimental design is a split plot and therefore the effect of accession was tested over accession × plot (nested within treatment); the treatment effect was tested over the plot effect; the plot effect was tested over the error mean square; and the accession × treatment effect was tested over the accession × plot interaction (SYSTAT, 1999). Tests were performed first by using a nominal α-value of 0.05, and then corrected for multiple tests using a sequential Bonferroni correction (Rice, 1989).

Univariate ANOVA contrasts were carried out for those traits with a significant accession or accession × treatment effect (genetic variation for trait means or plasticities among accessions). These contrasts were informed by the cpDNA phylogeny (Fig. 1). Contrasts were made by comparing accessions according to their grouping on the phylogeny: for example, Norway 1643 was compared with Norway 1436 because they formed a distinct clade; the average means or plasticities of these accessions were then compared with the corresponding value for Finland 1160, which form a clade with the two Norway haplotypes; and so on. For the polytomies, the unresolved accessions were contrasted with each other (e.g. Sweden 1352 with Finland 1144), then *each* was contrasted with those accessions making up the sister branch (e.g. Sweden 1352 with Denmark 1288, Finland 1160, Norway 1643, Norway 1436 and *A. suecica*). Again, the significance levels for the ANOVA contrasts were corrected using a sequential Bonferroni adjustment.

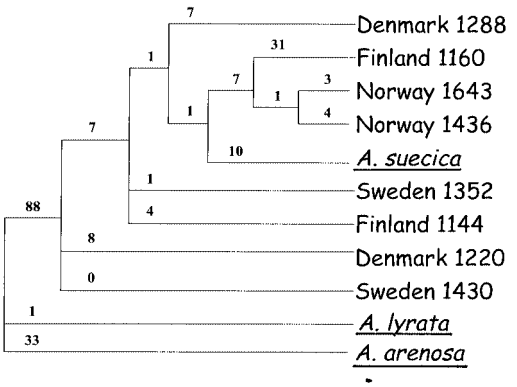

Fig. 1. Phylogenetic tree of *Arabidopsis thaliana* haplotypes, *A. suecica*, *A. arenosa* and *A. lyrata* obtained using cpDNA sequences and the maximum likelihood method. Numbers indicate number of point mutations supporting each node.

Least square means were plotted as bar diagrams for each trait that was significant for the accession effect. The ANOVA contrasts highlighted which of the accessions were significantly different for their least square means. Also, bar diagrams showing the plasticity of each trait for each accession were plotted for all traits for which the accession × treatment effect was significant. These plasticities were calculated by subtracting the mean value for each character in the short daylength treatment from the mean value of that character in the long daylength treatment. Positive and negative values of the bars indicate the direction of the corresponding plasticities. Again, ANOVA contrasts were used as a guide to which haplotypes showed significantly different plasticities from each other.

Bivariate analyses were carried out using COMPARE, a package of programs used to examine comparative data while taking into account the phylogenetic context (Martins, 1995). COMPARE is based on Felsenstein's PHYLIP program, which implements an algorithm that calculates the character values at each node along a phylogeny, thereby making comparisons of different traits independent from common descent (Felsenstein, 1985). These phylogenetically independent contrasts (not to be confused with the phylogenetically informed ANOVA contrasts discussed above) showed whether or not the plasticities or the means of different haplotypes are significantly correlated once the common ancestry of the accessions is factored out. COMPARE requires bifurcating trees, thus the polytomies within the phylogenetic tree were assigned extremely short branch lengths (0.0001); all other branch lengths were set to a value of 1.

Three correlation tables were created from the phylogenetic contrasts: (1) between all pairwise combinations of across-treatment trait means (to quantify co-evolution of trait means); (2) between all pairwise combinations of plasticities (co-evolution of plasticities); and (3) between the across-environment mean and the corresponding plasticity for all traits (co-evolution of the mean and plasticity of the same trait). Significant correlations indicated those combinations of traits that co-evolved along the phylogeny, while taking into account the relatedness of the accessions used in the experiment.

Multivariate matrix comparison tests were conducted to test specific hypotheses about the evolution of among-accession differences in trait means and plasticities. We used non-

parametric Mantel tests (Manly, 1985) carried out with the NTSYS-pc2 software package (Rohlf, 1998). Mantel tests assess the relationship between two independently derived matrices and compute their correlation and Mantel test statistic, Z. The observed Z value is then contrasted with an empirical distribution of Z values obtained by 1000 random permutations of one of the two matrices being compared. The following matrices were contrasted with one another: a flowering time mean difference matrix and a flowering time plasticity difference matrix against: (1) a phylogenetic distance matrix [based on the uncorrected dissimilarity (D) or p-distance; PAUP version 4.0b2]; (2) a matrix of difference in latitude among accessions; and (3) a geographic distance matrix (based on calculations from http://www.indo.com/distance/). Because the exact location of the Finland 1160 haplotype is not known, we created two latitude matrices and two geographic location matrices assuming Finland 1160 was collected at Espoo, Finland (approximately 60°N/25°E) or at Puolanka, Finland (65°N/28°E); the results of the Mantel tests were not affected by the precise location of this accession.

RESULTS

Tree estimation

The three intergenic regions analysed were combined to produce a 2100 bp sequence that included 38 in/dels. Sequence variation was high among accessions of *A. thaliana* (0.2–2.3% between pairs of haplotypes) and between the ingroup and outgroups (3.4–4.1% and 5.7–6.3% for comparisons with *A. arenosa* and *A. lyrata*, respectively). The outgroup species *A. arenosa* and *A. lyrata* were distinguished from the *A. thaliana* clade (including *A. suecica*) by 54–62 and 83–92 substitutions respectively, as well as a 182 bp in/del (present in both outgroup accessions but missing in *A. thaliana*). The observed transition bias for all haplotypes was close to 2 : 1 (ts : tv = 2.13 : 1).

Likelihood scores were highest for two of the most complex of the 16 models of sequence evolution, which included six substitution types and four rate categories (General-time-reversible models: Yang *et al.*, 1994) and only differed with respect to whether the proportion of sites able to accept substitutions was assumed to be zero or was estimated (likelihood scores = –3891.89 and –3890.21 for models assuming zero and an estimated proportion of invariant sites, respectively). These models were not significantly different from each other (likelihood ratio test $P > 0.10$, 1 d.f.), but both differed from the next best model of sequence evolution (likelihood ratio test $\chi^2 = 157.93$, $P \ll 0.001$, 1 d.f. and $\chi^2 = 159.61$, $P \ll 0.000$, 1 d.f. for zero and estimated invariants, respectively) (Yang *et al.*, 1994). Assumptions corresponding to the Jukes-Cantor model (JC, one substitution type and equal rates of evolution: Jukes and Cantor, 1969) and Kimura's two-parameter model (K2P, two substitution types and equal rates of evolution: Kimura, 1980) produced the lowest scores (–4113.92 and –4112.70 for JC and K2P, respectively). A cladistic analysis of all accessions using all four of these models resulted in the same single tree that contained two polytomies (ts : tv = 1 : 1; Fig. 1) and a likelihood score that was higher than the likelihood scores obtained for any of the trees generated with maximum parsimony (likelihood ratio test for the closest comparison: $\chi^2 = 5.49$, $P < 0.025$, 1 d.f.). The tree pictured in Fig. 1 was also obtained when the transition : transversion bias was assumed to be close to the observed level (ts : tv = 2 : 1) or higher than the observed level (ts : tv = 5 : 1).

Variation and evolution of reaction norms

We tested for the effects of four factors on the observable variation in the eight traits considered by using a mixed-model analysis of variance (Table 1): accession (genetic differences among accessions or species), treatment (overall phenotypic plasticity), plot (nested within treatment, indicating micro-environmental heterogeneity within the experimental set-up) and accession × treatment (variation for plasticity among haplotypes or species). Plots pooled three trays from a single shelf in a single treatment; we were unable to use individual trays due to some missing data points. There was a significant difference in the trait means of the haplotypes (accession effect) for all of the eight traits. The treatment effect was significant for two traits: rosette leaf number at bolting and days to bolting. Significant variation among haplotypes for plasticity (accession × treatment interaction) was found for two traits: number of basal stems and number of basal fruits. There were no significant micro-environmental effects (plot effect).

Phylogenetically informed (i.e. based on the structure of the cladogram in Fig. 1) ANOVA contrasts for across-treatment trait means were carried out for all eight traits with a significant accession effect (Table 2). The trait means for each accession are

Table 1. Analysis of variance of accession, treatment, plot (nested within treatment) and accession × treatment effects

Trait	Accession (10 d.f.)	Treatment (1 d.f.)	Plot (treatment) (10 d.f.)	Accession × treatment (10 d.f.)	Error (209–218 d.f.)
Rosette leaf number at bolting (square root)	98.17 **(0.0000)**	25.92 **(0.0069)**	0.18 (0.8079)	3.21 (0.0227)	1.13
Rosette diameter at bolting	203.22 **(0.0000)**	122.38 (0.1494)	23.39 (0.0189)	18.84 (0.0157)	6.13
Days to bolting (square root)	640.55 **(0.0000)**	86.06 **(0.0013)**	0.11 (0.8850)	3.50 (0.0118)	1.07
Main stem length	123557.72 **(0.0000)**	1991.45 (0.4287)	2055.24 (0.4930)	2604.33 (0.7650)	4081.58
Number of basal stems (square root)	4.27 **(0.0000)**	2.03 (0.4743)	2.65 (0.0328)	2.07 **(0.0000)**	0.23
Days to first seed set (square root)	11.35 **(0.0000)**	7.01 (0.0229)	0.17 (0.6922)	0.55 (0.2467)	0.39
Main fruit number	57823.11 **(0.0000)**	33949.17 (0.1028)	4112.78 (0.2716)	7138.25 (0.1847)	4523.55
Basal fruit number	5426.96 **(0.0000)**	13986.92 (0.0229)	332.26 (0.7629)	6219.68 **(0.0000)**	581.21

Note: Mean squares and associated *P*-values (in parentheses) are reported (d.f. = degrees of freedom). Transformations of original data to improve normality are indicated in parentheses. The two treatments were 14 h and 22 h light per day respectively. **Boldface** indicates *P*-values significant after a sequential Bonferroni correction.

Table 2. Phylogenetically informed ANOVA contrasts for trait means (for characters with a significant accession effect in Table 1)

Contrast	Rosette leaf number at bolting	Rosette diameter at bolting	Days to bolting	Main stem length	Number of basal stems	Days to first seed set	Main fruit number	Basal fruit number
Norway 1643 *vs* Norway 1436	0.97 (0.2770)	1.24 (0.6339)	0.01 (0.9177)	3084.18 (0.2951)	0.15 (0.6530)	1.70 (0.0512)	20174.64 (0.0114)	0.02 (0.9967)
Norway 1643 + Norway 1436 *vs* Finland 1160	141.31 **(0.0000)**	141.26 **(0.0000)**	207.55 **(0.0000)**	544202.47 **(0.0000)**	1.29 (0.1951)	1.58 (0.0596)	202842.03 **(0.0000)**	1298.81 (0.3046)
Norway 1643 + Norway 1436 + Finland 1160 *vs* *suecica* 3220	99.92 **(0.0000)**	71.69 **(0.0004)**	2.86 (0.0803)	13902.69 (0.0269)	0.03 (0.8468)	41.57 **(0.0000)**	13814.39 (0.0358)	62.02 (0.8223)
Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 *vs* Denmark 1288	111.42 **(0.0000)**	25.93 (0.0304)	30.95 **(0.0000)**	279387.16 **(0.0000)**	15.41 **(0.0000)**	2.00 (0.0344)	77348.00 **(0.0000)**	7114.43 (0.0169)
Finland 1144 *vs* Sweden 1352	44.23 **(0.0000)**	42.00 (0.0060)	25.83 **(0.0000)**	186.84 (0.7964)	0.07 (0.7608)	4.03 **(0.0028)**	33285.33 **(0.0012)**	168.75 (0.7110)
Sweden 1352 *vs* Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	8.82 **(0.0012)**	12.45 (0.1324)	18.52 **(0.0000)**	9794.56 (0.0628)	0.06 (0.7809)	2.02 (0.0336)	45513.16 **(0.0002)**	176.61 (0.7047)
Finland 1144 *vs* Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	30.43 **(0.0000)**	22.33 (0.0443)	4.79 (0.0241)	6684.25 (0.1238)	0.33 (0.5101)	1.23 (0.0964)	213.30 (0.7932)	8.77 (0.9327)
Denmark 1220 *vs* Sweden 1430	45.62 **(0.0000)**	2.08 (0.5375)	27.90 **(0.0000)**	3616.60 (0.2570)	0.53 (0.4065)	6.03 **(0.0003)**	9221.78 (0.0859)	0.0187 (0.9969)
Denmark 1220 *vs* Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	190.80 **(0.0000)**	14.79 (0.1011)	102.55 **(0.0000)**	148518.92 **(0.0000)**	5.61 (0.0073)	7.32 **(0.0001)**	99746.90 **(0.0000)**	2271.41 (0.1750)

Table 2. *continued*

Contrast	Rosette leaf number at bolting	Rosette diameter at bolting	Days to bolting	Main stem length	Number of basal stems	Days to first seed set	Main fruit number	Basal fruit number
Sweden 1430 *vs* Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	26.19 **(0.0000)**	3.77 (0.4067)	10.93 **(0.0007)**	94024.35 **(0.0000)**	1.95 (0.1111)	0.29 (0.4188)	36254.79 **(0.0007)**	2211.17 (0.1808)
arenosa vs lyrata 3219	1.03 (0.2619)	1106.67 **(0.0000)**	11.34 **(0.0006)**	47894.52 **(0.0001)**	1.58 (0.1511)	43.80 **(0.0000)**	18295.86 (0.0159)	910.05 (0.3900)
lyrata 3219 *vs* Sweden 1430 + Denmark 1220 + Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	199.21 **(0.0000)**	9.77 (0.1823)	200.97 **(0.0000)**	4638.05 (0.1995)	15.06 **(0.0000)**	9.51 **(0.0000)**	5263.25 (0.1937)	29541.21 **(0.0000)**
arenosa vs Sweden 1430 + Denmark 1220 + Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	229.84 **(0.0000)**	1671.69 **(0.0000)**	88.82 **(0.0000)**	125745.99 **(0.0000)**	4.60 (0.0149)	32.33 **(0.0000)**	61822.49 **(0.0000)**	16535.66 **(0.0003)**

Note: Each contrast tests the hypothesis of variation for trait means among haplotypes that are part of the particular subclade described in the first column. MS and *P*-values (in parentheses) are reported. **Boldface** indicates contrasts significant after a sequential Bonferroni correction.

plotted in Figs 2–4. The contrasts for rosette leaf number at bolting were significant in 11 of 13 cases. *A. lyrata*, *A. arenosa* and *A. suecica* had significantly different means for rosette leaf number and showed distinctly lower values for this trait compared to all *A. thaliana* accessions (Fig. 2a). The highest number of leaves was found in both Danish and Finnish accessions (despite their phylogenetic distance) as well as in one of the Swedish accessions. Contrasts for rosette diameter at bolting were significant in only four instances, and *A. arenosa* showed a much higher value than all other accessions

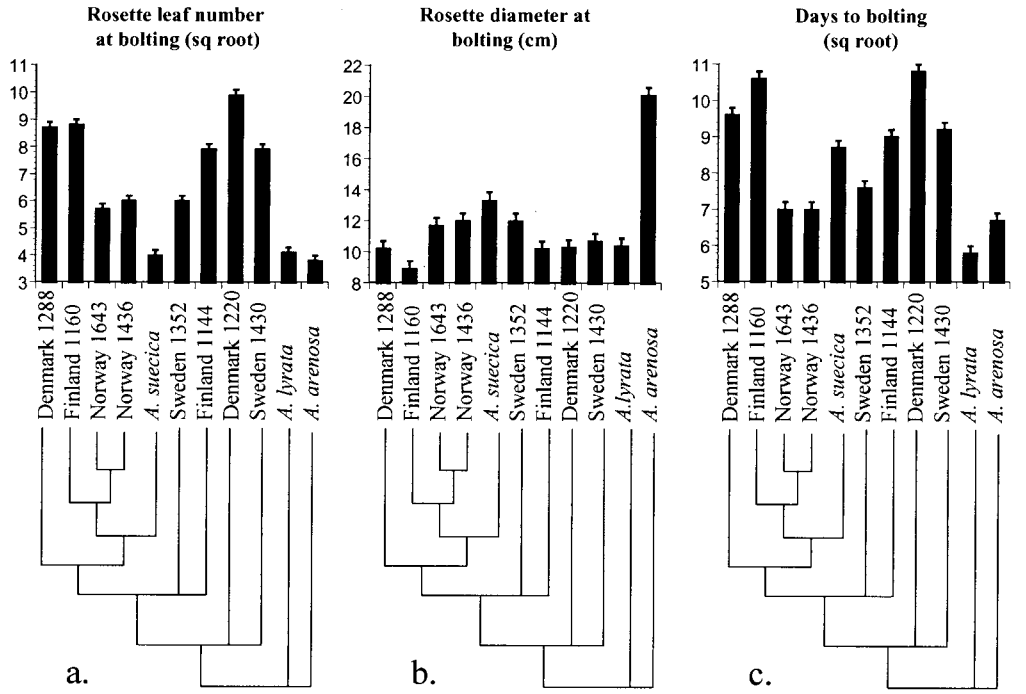

Fig. 2. Across-environment means for vegetative-stage characters (leaf number, rosette diameter and days to bolting), superimposed to the cladogram for comparative purposes.

(Fig. 2b). For the remaining vegetative-stage trait, days to bolting, 10 of 13 contrasts were statistically significant. *A. lyrata* and *A. arenosa* were characterized by the lowest trait values, with the Finnish and Danish and one Swedish accessions flowering late (Fig. 2c). The two closely related Norwegian accessions were indistinguishable for all vegetative traits.

Among the reproductive-phase characters, six of 13 contrasts were significant for main stem length (Table 2), with accessions characterized by dramatic differences in this trait when data were averaged across treatments (Fig. 3a). The two Norwegian accessions had much greater stem length than any other entry and were once again very similar to each other. Both Danish haplotypes were characterized by the shortest stems (again, note that these two are phylogenetically distant), together with a Finnish and a Swedish accession. Two contrasts were significant for number of basal stems, and a Danish accession together with the two outgroups had the greatest number of basal stems (Fig. 3b). There were seven significant contrasts for days to first seed set; no particular trend was discernible from the corresponding plot, with the exception of a longer time for seed maturation in both *A. suecica* and *A. arenosa* (Fig. 3c).

As far as reproductive components of fitness are concerned, seven of the 13 contrasts for main fruit number were significant (Table 2), with an overall pattern closely matching the one discussed for stem length (except that the two closely related Norwegian accessions

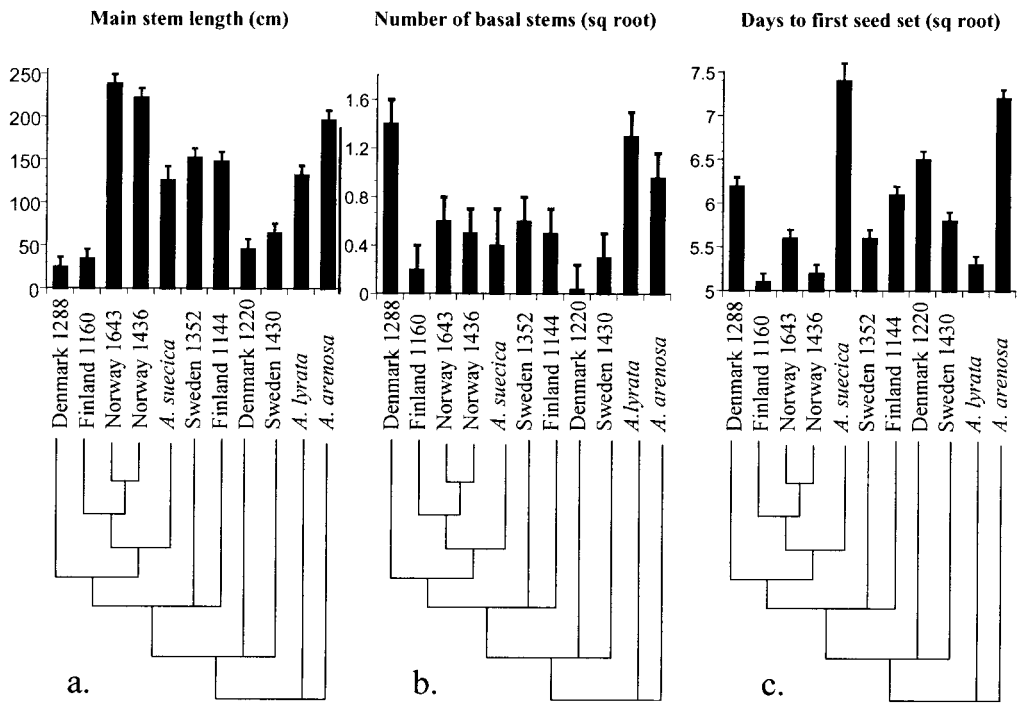

Fig. 3. Across-environment means for reproductive-stage characters (main stem length, number of basal stems and days to first seed set), superimposed to the cladogram for comparative purposes.

differed in fruit number but not in stem length) (Fig. 4a). For basal fruit number, only two of the 13 contrasts were significant, both of which involved very basal nodes in the phylogeny. *A. lyrata* and *A. arenosa* had a significantly higher number of basal fruits than any other accession, with the partial exception of one Danish entry (Fig. 4b). The overall pattern for this trait closely resembled the one for number of basal stems discussed above.

Phylogenetically informed ANOVA contrasts for trait plasticities were carried out for the two characters with a significant accession × treatment effect (Table 3). The plasticities (difference between character mean under short *vs* long daylength) for each accession are depicted in Fig. 5. Two of 13 contrasts were significant for basal stem number plasticity. *A. arenosa* and *A. lyrata*, and to a lesser extent *A. suecica*, and the two Norwegian *A. thaliana* haplotypes produced more basal stems under long days (Fig. 5a); three accessions (Sweden 1352, Finland 1144 and Sweden 1430) had more stems under short days; the rest showed virtually no plasticity. We also found the same two contrasts (basal to the cladogram) to be significant for basal fruit number plasticity. The pattern was similar to the previous character, with the distinction that most plasticities were more attenuated, with only the two outgroups and to a much lesser extent a Swedish and a Norwegian accession departing from a flat reaction norm (in opposite directions: Fig. 5b).

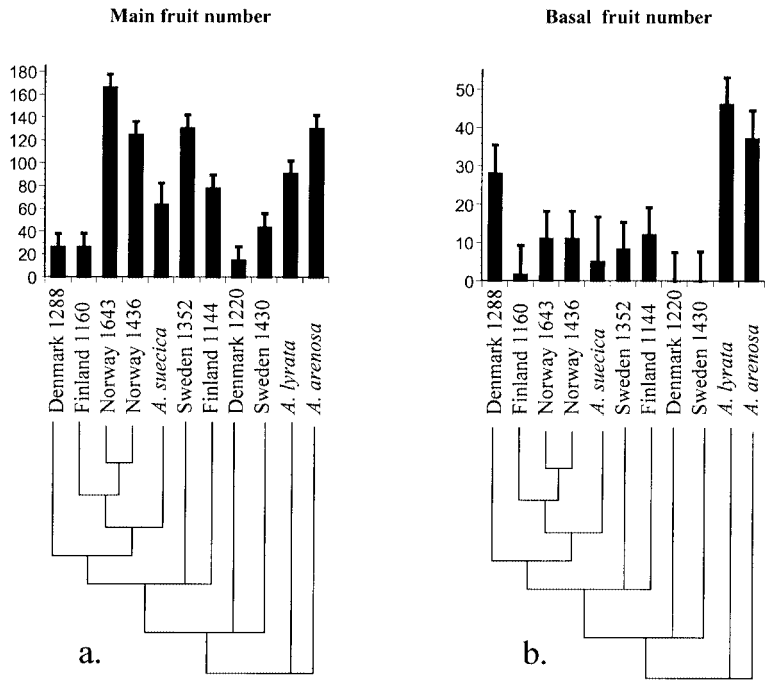

Fig. 4. Across-environment means for fitness characters (main fruit and basal fruit production), superimposed to the cladogram for comparative purposes.

Covariation and co-evolution of reaction norms

Using the COMPARE program of Martins (1995), we obtained three phylogenetically corrected correlation matrices for all traits measured: one comparing across-treatment means, one comparing plasticities, and one comparing across-treatment means and plasticities of the same trait. These matrices summarize the patterns of co-evolution between character means, character plasticities, and means and plasticities of the same trait. Eight of the 28 across-treatment mean correlations were significant; three more were just below nominal significance (Table 4). There was one positive correlation between vegetative characters, relating the number of rosette leaves to the time to bolting; two correlations were significant and positive among reproductive traits, main stem length versus main fruit number and number of basal stems versus number of basal fruits. The remaining correlations were mostly negative (with the exception of rosette diameter and basal fruit number) and related vegetative to reproductive traits.

The second correlation matrix shows correlations between trait plasticities for all measured characters once the phylogenetic relationships are taken into account. Five of the 28 correlations were significant and one more was just below the significance threshold (Table 5). There was a negative correlation within vegetative traits, between plasticity of rosette diameter and plasticity of bolting date; we found three significant correlations among reproductive characters, all positively relating the plasticities of main stem length and days to first seed set, main stem length and basal fruit number, and number of basal

Table 3. Phylogenetically informed ANOVA contrasts for trait plasticities (for characters with a significant accession × environment effect in Table 1)

Contrasts	Number of basal stems	Basal fruit number
Norway 1643 *vs* Norway 1436	0.40 (0.4589)	1230.19 (0.3014)
Norway 1643 + Norway 1436 *vs* Finland 1160	0.65 (0.3424)	111.92 (0.7550)
Norway 1643 + Norway 1436 + Finland 1160 *vs suecica* 3220	0.03 (0.8743)	75.52 (0.7977)
Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 *vs* Denmark 1288	0.57 (0.3740)	205.85 (0.6722)
Finland 1144 *vs* Sweden 1352	0.24 (0.5665)	374.08 (0.5684)
Sweden 1352 *vs* Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	3.41 (0.0308)	1036.54 (0.3427)
Finland 1144 *vs* Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	1.52 (0.1483)	64.20 (0.8132)
Denmark 1220 *vs* Sweden 1430	0.47 (0.4219)	0.01 (0.9976)
Denmark 1220 *vs* Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	0.20 (0.6012)	28.69 (0.8745)
Sweden 1430 *vs* Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	1.81 (0.1151)	27.32 (0.8775)
arenosa vs lyrata 3219	0.08 (0.7403)	442.77 (0.5350)
lyrata 3219 *vs* Sweden 1430 + Denmark 1220 + Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	6.23 **(0.0037)**	38302.03 **(0.0000)**
arenosa vs Sweden 1430 + Denmark 1220 + Finland 1144 + Sweden 1352 + Norway 1643 + Norway 1436 + Finland 1160 + *suecica* 3220 + Denmark 1288	7.91 **(0.0011)**	26899.18 **(0.0000)**

Note: Each contrast tests the hypothesis of variation for plasticity among haplotypes that are part of the particular subclade described in the first column. MS and *P*-values (in parentheses) are reported. **Boldface** indicates contrasts significant after a sequential Bonferroni correction.

stems and basal fruit number. Only one correlation was significant and negative between plasticities of vegetative and reproductive traits, linking days to bolting and main fruit number.

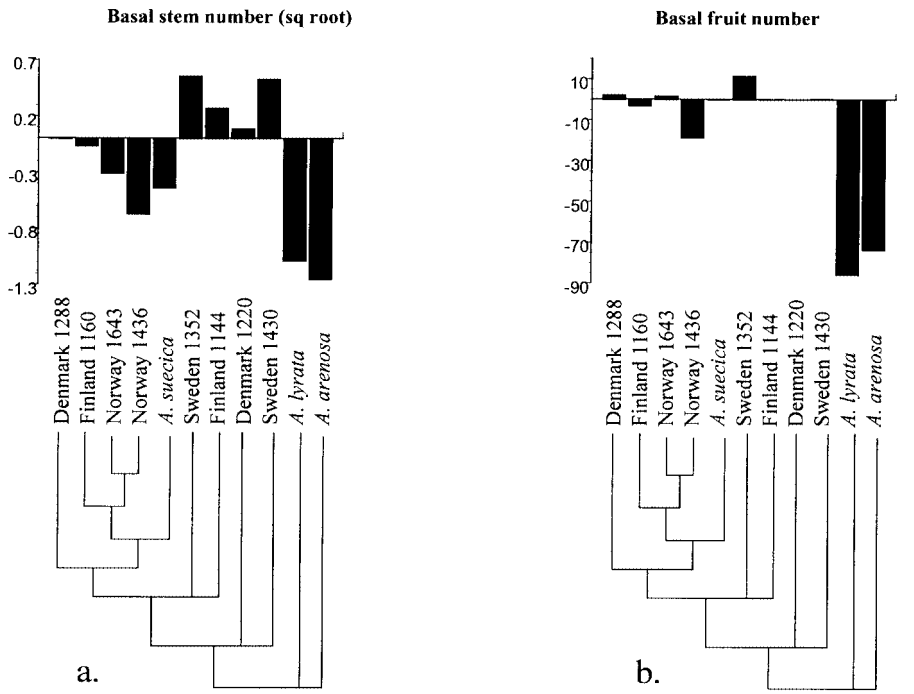

Fig. 5. Plasticities (phenotype under short daylength minus phenotype under long daylength) of traits with a significant accession × environment interaction (numbers of basal stems and basal fruits), superimposed to the cladogram for comparative purposes.

Table 4. Phylogenetically corrected correlations among the across-environment means of all traits

	Rosette leaf number at bolting	Rosette diameter at bolting	Days to bolting	Main stem length	Number of basal stems	Days to first seed set	Main fruit number	Basal fruit number
Rosette leaf number at bolting	1.0000							
Rosette diameter at bolting	0.6225	1.0000						
Days to bolting	***0.8801***	–0.6187	1.0000					
Main stem length	***–0.7991***	0.4245	***–0.8873***	1.0000				
Number of basal stems	–0.1625	0.6116	–0.3681	0.0174	1.0000			
Days to first seed set	–0.4356	0.4940	–0.1325	0.2569	–0.1385	1.0000		
Main fruit number	**–0.7109**	0.4205	***–0.8995***	***0.8882***	0.2714	–0.0565	1.0000	
Basal fruit number	–0.2401	***0.8028***	–0.4371	0.2761	***0.7719***	0.1052	0.3718	1.0000

Note: Critical values of the correlation coefficient: $r \geq 0.765$ ($P = \mathbf{0.01}$); $r \geq 0.632$ ($P = \mathbf{0.05}$).

The third matrix summarizes correlations between across-treatment means and plasticities of the same traits (Table 6). Two of the eight correlations were significant and three more were just below significance. Both nominally significant correlations were negative

Table 5. Phylogenetically corrected correlations among trait plasticities

	Rosette leaf number at bolting	Rosette diameter at bolting	Days to bolting	Main stem length	Number of basal stems	Days to first seed set	Main fruit number	Basal fruit number
Rosette leaf number at bolting	1.0000							
Rosette diameter at bolting	0.0047	1.0000						
Days to bolting	0.2972	***–0.8034***	1.0000					
Main stem length	–0.3923	–0.0150	0.1505	1.0000				
Number of basal stems	0.1841	–0.1767	0.0904	0.4458	1.0000			
Days to first seed set	–0.0708	–0.1459	0.3770	**0.6666**	0.1505	1.0000		
Main fruit number	–0.4865	0.4777	***–0.7810***	–0.0168	0.0878	–0.5794	1.0000	
Basal fruit number	0.0048	0.3119	–0.2996	**0.6471**	**0.7564**	0.2041	0.3614	1.0000

Note: Critical values of the correlation coefficient: $r \geq 0.765$ ($P = \mathbf{0.01}$); $r \geq 0.632$ ($P = \mathbf{0.05}$).

Table 6. Phylogenetically corrected correlations between across-environment means and their respective plasticities

	Correlation
Rosette leaf number at bolting	–0.2187
Rosette diameter at bolting	–0.5224
Days to bolting	***–0.8315***
Main stem length	–0.2929
Number of basal stems	0.0532
Days to first seed set	0.5163
Main fruit number	–0.5621
Basal fruit number	**–0.6565**

Note: Critical values of the correlation coefficient: $r \geq 0.765$ ($P = \mathbf{0.01}$); $r \geq 0.632$ ($P = \mathbf{0.05}$).

(and so were two of the remaining three), inversely relating mean and plasticity of bolting time and mean and plasticity of basal fruit number.

Flowering time, geography, latitude and phylogeny

We ran a series of Mantel tests to determine whether there is an association between the focal trait of flowering time (mean or plasticity), a major determinant of the life history of *Arabidopsis*, and the phylogenic distance, latitudinal differential or geographic distance of the accessions (Table 7). None of the six tests showed a significant correlation between the matrices.

DISCUSSION

We used the phylogenetic comparative method to examine the evolution of reaction norms to photoperiod in a weedy annual. The aim was to gain insights into the evolution of

Table 7. Mantel tests for the association between differences in means or plasticity of days to bolting among accessions and their distance in degrees of latitude, kilometres or number of mutations across the phylogeny

Comparison	r	P_{1000}
Mean *vs* phylogeny	–0.16	0.1948
Mean *vs* latitude	–0.05	0.4066
Mean *vs* geographic location	–0.21	0.1419
Plasticity *vs* phylogeny	+0.03	0.3916
Plasticity *vs* latitude	+0.26	0.0599
Plasticity *vs* geographic location	+0.20	0.1578

Note: Each test was carried out with 1000 random permutations of one matrix while holding the other constant. The value of the calculated correlation coefficient (r) between each pair of matrices and the P-value obtained by randomization (P_{1000}) are reported.

the main components of reaction norms – the across-environment mean and the degree and pattern of phenotypic plasticity. Such studies can help to reveal the relative importance of historical patterns of common descent and deterministic forces such as natural selection and genetic constraints (Albuquerque *et al.*, 1997; Morand *et al.*, 1997; Huber *et al.*, 1998). For many species of animals, response to daylength is important in the timing of reproductive events (e.g. Gomi, 1996; Smith *et al.*, 1997; Gulledge and Deviche, 1998). Plants also use daylength cues for the timing of life-history events, but in their case daylength also changes the total amount of photosynthetically active radiation, thereby creating a complex relationship between seasonal cues and resource availability.

Variation and evolution of trait means and plasticities

We found variation among accessions for trait means and, to a much lesser extent, for trait plasticities (Table 1). Since these are differences among geographically separated accessions, they represent the outcome of past selective forces or of constraints rather than an estimate of currently available variation for future responses to selection (Armbruster and Schwaegerle, 1996; Schluter, 1996; Merila and Bjorklund, 1999). The latter can be assessed through selection experiments or estimates of genetic variation within each accession.

Some of the traits showed parallel changes across the phylogeny (Figs 2–5). These patterns were confirmed by an analysis of trait pairwise correlations (Tables 4–6; see below). Interestingly, it appears that trait means and plasticities followed very distinct paths through evolutionary time. Trait means have changed dramatically throughout the phylogeny with no particular regard for the genetic relatedness or geographical proximity of the accessions studied. A consistent exception to the lack of a geographical pattern is represented by the two Norwegian accessions, which showed essentially identical profiles for every character (Figs 2–5). These haplotypes, however, are also very closely related phylogenetically, making it impossible to determine if historical events or mechanistic forces have been responsible for their phenotypic similarity. The phylogenetically informed contrasts (Tables 2 and 3) pointed out that there was no tendency for change in trait means to be concentrated towards

the base or the tips of the phylogeny, again implying rapid shifts in character means throughout the history of the group. On the other hand, it is important to realize that some trait means indeed changed much more often than others throughout the phylogeny. For example, rosette leaf number and bolting time were significantly different in 11 and 12 of the 13 contrasts respectively, indicating a very rapid and widespread evolution of these traits. Number of basal stems and their fruit production, however, were different among only a few of the nodes of the phylogeny, suggesting a much more conservative evolutionary pattern. In this respect, it is interesting to note that bolting and leaf number are essential (and correlated; see below) aspects of the life history of *Arabidopsis*, which mark a complex switch between the vegetative and the reproductive phases of the life cycle (Schultz and Haughn, 1993). Many genes have already been identified as controlling these twin traits (Coupland, 1995) and allelic variation at some of these loci has been found in natural populations (Kuittinen *et al.*, 1997). Furthermore, some of the naturally segregating alleles at two of the candidate loci have actually been shown to increase the sensitivity of *Arabidopsis* to daylength (Alonso-Blanco *et al.*, 1998).

In stark contrast to the evolutionary flexibility of character means, trait plasticities in response to daylength appeared to be highly conserved throughout the phylogeny with only two (related; see below) characters showing significant variation across nodes (Table 3, Fig. 5). Interestingly, the plasticities of both basal stem production and basal fruit number only changed towards the very base of the phylogeny, with the two outgroups displaying a much higher (and similar) plasticity than any of the *A. thaliana* accessions or the *A. suecica* hybrid. This may imply that *A. thaliana* has seen a single reduction in plasticity of branching pattern to daylength very early in its evolution, although confirmation of this hypothesis awaits a larger sample of intra-specific accessions than the one we have investigated. As far as we are aware, this is the first documented case of a radically distinct pattern of evolution of character means and their plasticities established within a phylogenetic framework.

Co-evolution of trait means and plasticities

We found a complex pattern of co-evolution of trait means (Table 4), not unlike other comparative studies published to date (e.g. Ackerly and Donoghue, 1998; Hodkinson *et al.*, 1998; Villar *et al.*, 1998). Most of the phylogenetically independent correlations among trait means were found to link vegetative-stage and reproductive-stage character means, most of which were negative. Furthermore, all significant correlations within either vegetative or reproductive set were positive. This suggests the existence of two distinct clusters of covarying traits, analogous to Berg's concept of 'correlation pleiades' (Berg, 1960; Armbruster *et al.*, 1999), with the two sets in this case linked by a potential trade-off between the vegetative and reproductive stages. In particular, it is interesting to note that an investment in more vegetative meristems during the vegetative phase (basal leaves) translated into a *decrease* in the size of the reproductive stem and into fewer fruits being produced. Numerous other studies have found a trade-off between vegetative and reproductive allocation (e.g. Hiura *et al.*, 1996; Worley and Harder, 1996; Sugiyama and Bazzaz, 1997).

A similar negative relationship existed between bolting date and the same two reproductive traits, which can be accounted for by the positive (and almost universal in *Arabidopsis*: Mitchell-Olds, 1996; Pigliucci *et al.*, 1998) correlation between leaf number

and bolting time. The only exception to the trade-off between vegetative and reproductive structures was the positive correlation between rosette diameter and (basal) fruit production. From a functional standpoint, this simply means that larger photosynthetic organs yield higher reproductive output. When one considers this relationship together with the trade-off between leaf number and reproduction, an interesting picture emerges whereby *Arabidopsis* is faced by a meristem limitation determined by a developmental constraint (Hempel, 1996) that requires allocation and life-history 'decisions'; this is, however, independent of size relationships between vegetative and reproductive organs, which are probably mediated by the quality of the environment and the availability of resources (Clauss and Aarssen, 1994; Pigliucci *et al.*, 1995). Houle (1991) pointed out the possibility of just such a relationship when discussing the make-up of genetic correlations and their relationship to trade-offs.

The positive significant correlations among reproductive traits are also explainable in terms of the general architecture of *Arabidopsis*: main stem length was related to main fruit number and number of basal stems was related to basal fruit production for two reasons. First, this plant shows very little variation in inter-node length throughout main and lateral branches (M. Pigliucci, unpublished data); second, basal branches only produce a small and relatively constant number of fruits because, under most environmental circumstances, they do not have enough time to elongate significantly before plant senescence or death (M. Pigliucci, personal observation). Therefore, the combination of an architectural constraint and the relative shortness of the life cycle determines a direct proportionality between stem and fruit production.

We found evidence that trait plasticities evolved largely in an independent fashion along the phylogeny (Table 5), with few correlations both within and between the vegetative and reproductive sets identified above. This is not necessarily the case, since another study conducted in our laboratory has shown a highly intricate pattern of plasticity correlations in the same accessions in response to light spectral quality (H. Pollard *et al.*, submitted). In fact, both low and high levels of integration among plasticities have previously been found (Schlichting, 1986, 1989). We detected a significant negative correlation between the plasticities of rosette diameter and days to bolting as well as between the plasticities of main fruit number and days to bolting. This means that the more haplotypes are responsive to daylength for their life-history schedule, the less responsive they are in terms of vegetative growth and reproductive output. Since bolting time is a pivotal life-history trait in *Arabidopsis*, this may indicate that flexibility in the timing of the switch between vegetative and reproductive phases ensures a relatively constant vegetative growth rate and reproductive output. This is in agreement with the observation that – among these three traits – the only one with a statistically significant treatment term in the ANOVA was bolting time. This kind of situation, in which plasticity for one trait leads to homeostasis of other traits, has been described, for example, in *Polygonum* by Sultan (1995) and was originally discussed by Bradshaw (1965) in the context of co-adaptation of different plasticities.

During the reproductive phase, the plasticities of three traits were all positively related: main stem length, number of basal stems and basal fruit production. The latter two characters were associated with each other because of the architectural constraint on stem elongation discussed above; since inter-node length is relatively constant and most of the basal branches are short, there has to be a direct proportionality between number of stems and fruit production no matter what the environment is. The relationship between

the plasticity of main stem size and the other two implies that conditions that elicit taller stems also tend to produce higher basal branching. This is in accordance with the findings of Hempel and Feldman (1994), who found that the basal inflorescences are initiated in *Arabidopsis* only after a given number of flowers (on the main stem) have matured and opened, consistent with their model of sequential initiation of flower and axillary primordia during the reproductive phase of this plant. However, it is also known that main stem length and branching patterns do not have to be related, since gibberellin-insensitive and -deficient mutants can uncouple these two traits (Ross *et al.*, 1997) and potentially their plasticities (since gibberellin is responsive to light conditions, and daylength in particular: King *et al.*, 1994; Fink *et al.*, 1997; Jakson and Thomas, 1997; Junttila *et al.*, 1997).

Another question related to evolution of character means and plasticities that has often been raised is whether the mean and plasticity of the same trait can evolve separately, as originally suggested by Bradshaw (1965) and implied by Schmalhausen (1949). Evidence that this is possibile comes from Schlichting and Levin (1986) and Zimmerman (1976); pertinent discussions are in Schlichting (1986) and to some extent in Via *et al.* (1995). The answer emerging from our data seems to be what Bradshaw predicted: it depends. Technically, only two of our mean–plasticity correlations were significant (Table 6), although another three were nearly significant out of a total of eight. Most of these correlations (and especially all the significant ones) were negative, implying that a reaction norm with a higher across-environment mean tended to be associated with less plasticity. This finding strengthens the conclusion that plasticities and grand means can be considered distinct characters, since the obvious expectation would be of a positive relationship between the two (because the higher the mean is on the environment–phenotype axis, the more 'space' there is for the reaction norm to have a steep slope). From both our study and the few other examples available in the literature, it is reasonable to conclude that plasticities and their trait means behave exactly as any other group of characters one might encounter: sometimes they are related, either because of a shared genetic machinery or because of their functional ecology, but the association is by no means automatically implied by the fact that these are two properties of the same reaction norm.

Association of flowering time with phylogeny, latitude and geographic location

We also focused attention on flowering (bolting) time because it is such a defining character of the life history and phenotype of *Arabidopsis* and because it is relatively well understood from both a molecular and organismal standpoint (Koornneef *et al.*, 1991; Bagnall, 1992; Lee *et al.*, 1993; Coupland, 1995; Pigliucci and Schlichting, 1998). We would expect the mean flowering time of this species to be adapted to the prevalent daylength at a given location, but it is possible to imagine that other conditions, such as availability of nutrients (Zhang and Lechowicz, 1994; Pigliucci and Schlichting, 1998) or density of neighbours (Myerscough and Marshall, 1973; Davis and Simmons, 1994), might interfere with any overall pattern of variation. Our understanding of the plasticity of flowering time is less complete to say the least, mostly because of the lack of field investigations on the ecology of *Arabidopsis* (Napp-Zinn, 1985; Thompson, 1994). In the case of daylength in particular, the simplest expectation is for the plasticity of flowering time to evolve mostly by non-deterministic forces such as random drift, since the progeny of a given plant will experience the same seasonal cycle of variation in daylength as their parent. The possible exception occurs if a given population goes through two life cycles in a given year, which has been

reported in a few localities (Thompson, 1994). However, note that Scandinavian haplotypes of *A. thaliana* are known for being almost exclusively winter annuals (Napp-Zinn, 1985).

The results of the Mantel tests comparing inter-accession differences in both mean and plasticity of time to bolting to differences in latitude, location and phylogenetic relatedness were all non-significant (Table 7). Several authors have also reported a lack of correspondence between phylogenetic distance and geographical location in *Arabidopsis*, consistent with the hypothesis of a recent and rapid, probably partially human-driven expansion of *Arabidopsis* throughout Europe (King *et al.*, 1993; Hardtke *et al.*, 1996; Innan *et al.*, 1997; Loridon *et al.*, 1997). The lack of correspondence between quantitative characters (mean or plasticity) and phylogenetic relatedness may suggest that these traits do not evolve by random drift, which probably governs the evolution of the semi-neutral or neutral molecular markers on which the phylogenetic hypothesis is based. The absence of a relationship between these quantitative traits and either geographical distance or latitude, however, does not point towards a simple adaptive scenario in which the reaction norms to daylength are tailored to large-scale geographical differences in environmental conditions, leaving open the possibility that selection acts at much smaller scales than the ones considered here (Stratton and Bennington, 1996). In fact, studies conducted by our group, on naturalized populations of *Arabidopsis thaliana* in Tennessee, have shown consistent selection for altering flowering time under field conditions (H.S. Callahan and M. Pigliucci, submitted). Of course, our results do not rule out a role of genetic constraints (caused by pleiotropy on genetic correlations or by some types of epistasis) on the evolution of these traits. This can only be addressed by within-population studies; however, such studies have shown significant genetic variation for reaction norms of flowering time (Pigliucci, 1997; Pigliucci and Schlichting, 1998).

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