Eco6logical and evolutionary genetics of Arabidopsis

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The crucifer Arabidopsis thaliana has been the subject of intense research into molecular and developmental genetics. One of the consequences of having this wealth of physiological and molecular data available, is that ecologists and evolutionary biologists have begun to incorporate this model system into their studies. Current research on A. thaliana and its close relatives ably illustrates the potential for synergy between mechanistic and organismal biology. On the one hand, mechanistically oriented research can be placed in an historical context, which takes into account the particular phylogenetic history and ecology of these species. This helps us to make sense of redundancies, anomalies and sub-optimals that would otherwise be difficult to interpret. On the other hand, ecologists and evolutionary biologists now have the opportunity to investigate the physiological and molecular basis for the phenotypic changes they observe. This provides new insight into the mechanisms that influence evolutionary change.

Biology is experiencing the age of model systems1. Our present understanding of genetics would have been very different if laboratories throughout the world had not agreed to concentrate their efforts on the fruit fly Drosophila melanogaster at the beginning of the century. Similarly, different branches of biology have adopted distinct organisms as being particularly convenient for the type of study at hand. As a consequence, we have considerable knowledge of the physiology of mice, the developmental biology of sea urchins, the molecular biology of Escherichia coli, and an understanding of disease resistance in tobacco. There are, of course, limits to this strategy of focusing on a reduced number of organisms. Although it has been possible to understand their biology in depth, it is also clear that we are forfeiting anything more than a superficial knowledge of the overwhelming majority of living organisms.

Fortunately, research in evolutionary biology can help to broaden the scope of our investigations. All organisms were derived from a single common ancestor, which is why they share the same genetic/molecular machinery. Thus, we can apply what we learn about a small number of organisms to the majority — at least as long as we do not extrapolate too far from our starting point in either ecological or phylogenetic space. The real question is how many model systems we need, and how far these generalizations can reasonably be extended.

Arabidopsis as a model system

Arabidopsis thaliana (L.) Heynh. is a small annual, white-flowered member of the Brassicaceae family, and is allied to other crucifers such as mustard, Brassica napus and broccoli. Arabidopsis thaliana was first adopted as a model system in plant genetics in the 1950s, largely as a target for mutagenesis studies2. More recently, A. thaliana has been the focus of physiological, developmental and genetic research that has made it the reference point for plant molecular biology3.
trends in plant science

From the standpoint of reductionist biology, the main advantages of *A. thaliana* reside in its small genome and reduced number of chromosomes; the ease with which it can be transformed; the fact that it is easy to grow at high density; and the fast life cycle of some of its ecotypes. The word ecotype is used loosely in the case of *A. thaliana*. The original use of the term was to indicate a genetically adapted ecolog-ical specialist, such as an alpine form, or a heavy metal tolerant form. *Arabidopsis thali-ana*’s ecotypes are usually distinguished into ‘early’ and ‘late’ flowering, depending on the length of the vegetative phase and type of phenology – spring ephemerals or winter annuals.

Recently, however, this species and its close relatives have also been the subject of ecological and evolutionary research. In particular, the time-4tal conditions, which have been the focus of cated by a variety of responses to environmen-tal conditions may favor one or an-other genotype, yielding an apparently random patchwork of genotypes across the landscape.

The complex-282es of what makes this astounding one of the rarest great genetic variation. Further-283more, quantitative genetic studies of several groups of plants have been distinguished. A genetic variation can vary considerably, thereby affecting the entire life cycle and reproduc-tiveness of the plant. At least four groups of lines of evidence point to higher values (around 1.2–2.2%) implying that it is a more interest-ing subject for studies on population genetics. Even the allozyme studies revealed 45 differ-ent multilocus genotypes based on seven poly-morphic loci when 470 individuals were sampled from 16 populations (with up to eight genotypes per population); these figures are not indicative of low genetic variation. Further-more, qualitative genetic studies of several growing seasons in *A. thaliana* have shown signifi-cant heritability and genetic variation both within and among populations. Also, mol-ecular studies using DNA sequencing of the inter-spacer (ITS) region of the chloroplast genome have revealed remarkable variation both among and within populations (M. Cruzan and M. Pigliucci, unpublished). Finally, pro-togyny may significantly contribute to out-putting in *A. thaliana* (M. Al-Shelhaz, pers. commun.). Clearly, there is the need to further as-8sess the mating system and population genetics of this species.

The complexities of weed ecology

*Arabidopsis thaliana* is an excellent example of what a weed can do from an ecological stand-point. Although it is rarely locally abundant (its populations seldom count more than a few scattered individuals or clumps), and it does not withstand competition with grasses or other tall vegetation, its geographical range is none-theless impressive. The northernmost limit of its distribution is northern Scandinavia, and the southermest tip of its range is in northern Africa and the Middle East. To date, and in spite of all we know about the physiology and molecular biology of *A. thaliana*, no system-atic investigation of what makes this astounding geographical range possible has identified such a ‘simple’ phenotype as having been undertaken. What is known, is that *A. thaliana* responds as are the genotypes to environmental variation over a wide range of spatial scales. When used for bioassays in a series of explant trials within an area of 50 m² of undisturbed forest, it was found that the environmental variance (meas-ured by the response of the plant) between sites, increased with distance. However, the correlation between sites decreased with sep-aration in the same fashion at all spatial scales examined. In other words, environments – as perceived by *A. thaliana* – display the same degree of complexity at all spatial scales.

An elegant experimental design was used to investigate the response of *A. thaliana* to selection over very short-spatial scales. Ran-dom combinations of seeds of three pheno-typically distinguishable lines were sown over and across a series of 5 × 1 m transects. If no selection occurred, one would expect to find random mixtures of mature plants at the end of the season. Instead, the results showed a very significant tendency for genotypes of the same kind to cluster together, and eliminate the other two kinds from each microsite. The success of a given genotype depended on the microsite, and the range of this effect was less than 50 m. This suggests that very localized environmental conditions may favor one or an-other genotype.
mental studies of more, because there have been few experi-
tal studies on the use of A. thaliana in studies on phenotypic plasticity – the way in which genotypes respond to heterogeneous environmental conditions13,19. The phytochrome genes in A. thaliana (which control response to light availability, light quality and photoperiod) are presently some of the best known examples of plasticity genes. A plasticity gene is a receptor of environmen-
tal change that effects a cascade of specific and fitness-enhancing developmental/pleno-
tic changes12. An investigation into the plasticity of a wild-type inbred line of several single-gene mutants representing potential plasticity genes is summarized in Fig. 1. This study demonstrates that the elimination of all of the phytochromes (which control the re-
sponse to canopy shade), transforms the plas-
tic wild type into a genotype that is totally unresponsive to this specific environmental change, although there is no effect on other kinds of plasticity. This lack of plasticity di-
rectly translates into a proportional reduction in reproductive fitness of the mutants (which are otherwise physiologically and develop-
mentally normal). This investigation demonstr-
ates the adaptive implications of phenotypic plasticity13.

Contrary to expectations based on its puta-
tive selfing habit, A. thaliana shows a tre-
mendous degree of variation for phenotypic plasticity to an array of environmental circum-
stances. A study of four populations (two early flowering and two late flowering ecotypes), re-
vealed significant responses to water, nutrient and light gradients, with significant genetic variation among populations for several traits in reaction to nutrient and light availability13. A larger survey of 26 populations found signifi-
cant population by environment interaction (i.e. genetic variation for plasticity) for five out of nine traits13. Size-fecundity relation-
ships also varied significantly in response to light, nutrient and pot volume gradients13. This latter study identified a minimum size thresh-
old that the plant has to reach to be able to flower. However, the value of the threshold is itself environmentally dependent (i.e. plastic).

Other researchers have used A. thaliana as a model system to explore the effects of her-
bivory14, and the interaction between her-
bivory and abiotic factors15. A poorly explored aspect of plasticity in A. thaliana (and in other plants and animals as well) is the interaction between development and environment. Eco-
logical studies have demonstrated develop-
mental stage-specific variation for plasticity14, and research carried out on mutants has pin-
pointed the complex and antagonistic mol-
ecular basis of developmental windows of plasticity15. Although a general framework for this kind of research has not yet emerged, it is a growing area and demonstrates the trend for a direct interaction between ecological and molecular genetics.

Quantitative genetics and constraints

Arabidopsis thaliana is surprisingly geneti-
cally variable, both at the DNA sequence and at the quantitative genetic level. This has af-
fected some interesting evolutionary studies of this plant. One old problem in evolutionary genetics is represented by dominance12,15. To understand the evolution of dominance we need studies addressing both its proximate causes (the genetic and physiological bases), and its ultimate causes (i.e. under what cir-
stances natural selection favors a certain degree of dominance). It has been shown that dominance of two mutations in A. thaliana that affect the production of abscisic acid is modulated by maternal effects16, which pro-
vides the proximate cause of dominance in this instance. This kind of work can be integrated into studies on the selective advantage of dif-
ferent degrees of dominance and of the evolu-
tionary dynamics of maternal effects, to yield insights into the broader question of why dominance evolves.

Because the distance over which the homog-
енizing effect of selection occurred turned out to be very small (with random distributions above that scale), one can reasonably infer that most of A. thaliana’s ecology is local. However, these experiments did not address the factors responsible for this extremely lo-
calized response to selection. To address this point, an investigation into the spatiotemporal effects of nitrogen and litter availability was carried out15. It was found that some English populations undergo two life cycles within a given year. Some plants flower in the spring (May–June), and their offspring do not go through dormancy, but germinate immediately and flower in late summer (September–Octo-
ber). The seeds of the summer generation then overwinter, to germinate the following spring. These investigations revealed that litter from the spring individuals fosters reproductive out-
put in the summer generation (mostly very small individuals responding to high density – a re-
sult of phenotypic plasticity). By contrast, the overwintering seeds cannot use the litter of the previous generation because it is washed away. However, because they are at a much lower population density, they produce plants with large rosettes, which accumulate photosynthate overwintering seeds cannot use the litter of the previous generation because it is washed away. However, because they are at a much lower population density, they produce plants with large rosettes, which accumulate photosynthate and yield a significant number of siliques. However, because they are at a much lower population density, they produce plants with large rosettes, which accumulate photosynthate and yield a significant number of siliques.

These experiments demonstrate how little we know about the ecology of A. thaliana. For example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological example, we do not know how widespread the two-generation cycle is, or which ecological
Recently, quantitative genetics has acquired a new tool with which to merge evolutionary and molecular genetics. It is now possible to use molecular markers to identify and map the positions of genetic regions affecting quantitative characters in natural populations. These elements are known as Quantitative Trait Loci (QTLs)27. QTL mapping has been used to study phenotypic plasticity, flowering time and life history28,29. However, a word of caution is necessary. If the stated goal is to investigate loci affecting life-history evolution in natural populations, it is inappropriate to compare artificially derived lines grown under continuous light30, as this is an unnatural condition. Care has to be taken to prevent our enthusiasm for interdisciplinary research and new techniques from getting in the way of our understanding of the underlying biology.

Another long-standing evolutionary problem is the question of constraints31,32. A very well known genetic constraint in A. thaliana is represented by a strong phenotypic and genetic correlation between flowering time and leaf production (i.e. between size and age at maturity)30,33. It is interesting that the intensity of this constraint depends on the genetic background, as well as on the environment in which the plant grows. It is also possible to break the constraint to some extent, and obtain plants with a very different combination of the two traits compared to the wild type. This can be achieved by inducing mutations in different backgrounds, and then comparing the position in phenotypic space of the mutants and the respective wild-type (Fig. 2). Such an approach represents one of the first experimental attempts to address the evolutionary problem of constraints.

The big picture: evolution and systematics of Arabidopsis

No matter how interesting a model system is from a molecular or even an ecological standpoint, the crucial test of its relevance from an evolutionary perspective is provided by the exploration of its phylogenetic neighborhood. Until recently, the systematics of Arabidopsis was confusing34, but several authors have concentrated on revising the situation, applying modern molecular and cladistic analyses to the group35-36.

Many of the species that used to be classified as being Arabidopsis have been considered as being fairly distant relatives, whereas taxa once in Arabis or Cardaminopsis are now thought of as being Arabidopsis in the strictest sense. The emerging phylogenetic picture of this genus immediately creates the opportunity to examine interesting questions from an evolutionary perspective. For example, A. thaliana is an annual, but several of its relatives are biennials or perennials. How often does a new flowering habit emerge through history? How are these evolutionary shifts related to the ecology, and therefore what are the selective pressures these plants experience? Furthermore, not all members of Arabidopsis are diploid. In fact, one of A. thaliana’s closest relatives is A. arenosa, which is a tetraploid. Recent research has confirmed an old speculation that A. thaliana and A. arenosa are respectively the female and male parent of a third taxon, A. suecica35,36. The wealth of molecular and physiological information available for A. thaliana opens up previously unforeseen possibilities for investigating the ecology and evolution of hybridization and polyploidy (as well as the evolution of mating systems), because A. thaliana is predominantly self-crossing, while the other two are outcrossing.

![Fig. 2. Relationship between bolting time and leaf number in Arabidopsis thaliana. The solid red circles represent the three wild-type (Dijon (D), Landsberg (L) and Wassilewskija (W)) populations. All other circles (diameter proportional to reproductive fitness, measured as the number of fruit produced) are EMS mutants from the (a) Dijon, (b) Landsberg and (c) Wassilewskija backgrounds. Note how genetic variation generated by mutation can dramatically alter the relationship between the two traits. Also, it is interesting to note that – contrary to expectation – many mutants display a higher reproductive fitness than the wild types. This effect might be because the three wild types have been selected for a very short lifecycle, which had the phenotypic effect of reducing the reproductive output (M. Pigliucci and N. Turner, unpublished).](image)

![Fig. 3. Evolution of phenotypic plasticity of leaf production in response to light quality in Arabidopsis and allied species. The length of the bars to the right of each taxon indicates the intensity of the plastic response: the color of the bar indicates distribution in Central Europe (yellow), Eastern Europe (red) and Northern Europe (blue). Hylandra (also known as A. suecica) indicates a hybrid between A. thaliana and (presumably) A. arenosa (belonging to the Cardaminopsis clade). The phylogenetic position of A. thaliana’s ecotypes is inferred from their genetic distances. Note how strong or weak plasticity has apparently evolved more than once in the whole group.](image)
The availability of a phylogeny of the Arabidopsis genus and its more or less distant relatives has also afforded some first-time comparisons analyses addressing evolutionary ecological questions. One such question concerns the evolution of phenotypic plasticity. For example, in the case of plasticity of leaf production, a high degree of plasticity occurs in some distant relatives outside Arabidopsis’s clade, but it is lost in early flowering taxa, only to re-evolve in late flowering ones (Fig. 3). It would be interesting to map the evolution of the known genes affecting flowering time upon the same cladogram.

Can we put everything together? Attempts to unify biology have characterized the history of this discipline throughout the 20th century. One of the most important results of this trend has been the neo-darwinian synthesis that led to modern evolutionary theory. Recently, many people have invoked a long-known cladogram for the basic sciences, and the relatively small techniques. However, given the limited funding for the basic sciences, and the relatively small number of scientists that study either molecular or evolutionary biology, a true synthesis seems possible— at least at present—only by concentrating on a few well-chosen organisms. Together with other classical model systems, such as E. coli and Drosophila, Arabidopsis and its relatives are clearly gaining permanent status as members of this intensively studied group.

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