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CANDIDATE GENES, QUANTITATIVE TRAIT LOCI, AND FUNCTIONAL TRAIT EVOLUTION IN PLANTS

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Two key characteristics of the neo-Darwinian synthesis in evolutionary biology have been its emphasis on the importance of mutations of small effect (micromutationism) and the view that studies of individual gene function shed relatively little light on evolutionary processes. Recent advances in molecular biology, however, have broken down many of the barriers between functional and evolutionary inquiry, opening the door to detailed studies of the genetic basis of functional trait evolution in plants. In this article, we review the insights into plant evolution that have been provided by molecular methods and address future research needs. Quantitative trait locus (QTL) mapping in crop and model plants has shown that individual loci often have large effects on trait variation, at variance with the micromutationist perspective. Evidence so far indicates that QTLs with large effects are also important in wild populations, underlying interspecific differences as well as intraspecific variation. Isolation of some of these QTLs, in particular for flowering time variation, has revealed a prominent role for regulatory genes known to function in regulation of flowering and exposed the complexity of regulatory processes. Preliminary evidence indicates that plant growth variation may be directly regulated rather than primarily the indirect result of selection on constituent processes. Future research should expand the number of traits that are intensively studied and make greater use of QTL mapping in wild plant taxa, especially those undergoing adaptive radiations, while continuing to draw on insights from model plants. Promising techniques include testing of candidate gene-trait associations in wild populations, genetic mapping in hybrid zones, and microarray analyses of gene expression.

Keywords: QTL, flowering time, candidate gene, adaptive evolution, regulatory genes.

Introduction

In *The Growth of Biological Thought*, Ernst Mayr devoted an extended discussion to the origins of the modern neo-Darwinian consensus in evolutionary biology and the conceptual unity it forged out of often-conflicting perspectives of the biometrically oriented "naturalist" and Mendelian camps (1982, pp. 540–570). Among the major issues that had to be resolved were questions about the nature of inheritance, the importance of continuous versus discontinuous variation to evolution, and whether novel mutations or natural selection on existing variation were the primary factors responsible for the origins of species. The population genetic concepts of R. A. Fisher (1918, [1930] 1992) provided a comprehensive framework to unite particulate inheritance with continuous trait variation in evolutionary thought and played a critical role in the resolution of the naturalist-Mendelian conflicts. Fisher demonstrated that many Mendelian factors of small effect, together with environmental causes of variability, could explain continuous trait variability in natural populations. Moreover, Fisher reasoned from a mathematical perspective that mutations of small effect would inevitably be the dominant factors in evolution because major mutations would almost always be deleterious ([1930] 1992, pp. 38–41).

Key aspects of the neo-Darwinian consensus, as described by Mayr, are (*a*) that evolution is a gradual continuous process of natural selection on genetic mutations of small effect and (*b*) that ecological processes acting on genetic variability within populations are the primary factors driving natural selection (1982, p. 567). Rival concepts, such as Goldschmidt's (1940) idea of speciation driven by major mutations that give rise to "hopeful monsters," were dismissed. Over the last few decades, however, some aspects of the neo-Darwinian consensus have come under renewed scrutiny. With the advent of molecular techniques in genetic studies, Allan Wilson's research group (King and Wilson 1975; Cherry et al. 1978) observed that human and chimpanzee protein and DNA sequences were surprisingly similar, given the pronounced morphological differences between the two species. They proposed that a small number of regulatory differences and chromosomal rearrangements might be largely responsible for morphological evolution. Meanwhile, Stephen Jay Gould (Gould and Lewontin 1979; Gould 1980) began to voice skepticism about both gradualism and pervasive adaptationism as explanations for speciation. Suddenly, it seemed that Goldschmidt's (1940) "hopeful monsters" had emerged from the closet to which they had been banished by the modern synthesis.

Debates during the 1980s over the strength of empirical evidence for the evolutionary importance of large-effect mutations (Gottlieb 1984; Coyne and Lande 1985) culminated in

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a cautious but technically thorough challenge to the micromutationist perspective by Orr and Coyne (1992). They concluded that Fisher's reasoning about the insignificance of major mutations was flawed on several grounds and that the evidence for micromutationism was surprisingly weak. Orr and Coyne did not declare support for a macromutationist viewpoint, but they did call for the use of emerging molecular marker techniques to study the roles of major versus minor genes in natural populations. More recently, Orr (1998) has expanded on Fisher's mathematical models and shown that the mutations fixed during adaptive evolution are likely to include some substitutions with large phenotypic effects.

Doebley and Lukens (1998) have hypothesized that mutations in the promoters of transcriptional regulatory genes, affecting their expression patterns rather than protein function, are more likely to produce large changes in plant morphology than are mutations in genes encoding structural or signaling proteins. According to their hypothesis, transcriptional regulators frequently control the expression of a number of genes functioning in a single pathway, so mutations affecting their levels of expression could produce large changes in a single trait with few side effects on other traits (pleiotropy).

Another characteristic of the modern synthesis has been a relative lack of emphasis on genetic mechanisms at the molecular level (Watt 2000). Evolutionary processes have been understood to operate somewhat independently from proximate functional mechanisms and thus to require different levels of biological inquiry (Mayr 1982, pp. 67–73). The components-of-variance techniques developed by Fisher, Wright, and Haldane for analyzing quantitative genetic phenomena and selection responses without reference to the functions of individual genes are still the primary tools of quantitative genetics (Falconer and Mackay 1996). The revolution in molecular genetics over the last few decades, however, has broken down some of the technical barriers between studies of gene function and trait evolution. Genetic mapping provides powerful insights into the genetic architecture of functional traits. Techniques of molecular biology have allowed isolation and functional characterization of individual genes and their products, providing a wealth of information on the genetic control of important developmental and metabolic processes. Genomic sequencing in a number of organisms, including *Arabidopsis thaliana*, and development of techniques to analyze expression of thousands of genes simultaneously provide further opportunities for new insights into evolutionary processes. So far, these molecular technologies have been applied primarily in organisms that serve as genetic model systems and in commercially important traits in economically valuable organisms such as crop plants. However, both the results emerging from these studies and the methods they use have great relevance for understanding evolution in natural populations.

In this review, we evaluate the current state of understanding for the genetic basis of functional trait evolution in plants and opportunities and prospects for future research. In particular, we address recent molecular insights into three key issues surrounding the genetics of plant evolution: (*a*) the extent to which major genes are involved in the evolution of trait differences within and between species, (*b*) the respective roles of mutations in structural versus regulatory genes in trait evolution, and (*c*) the nature of genetic regulation and functional interactions in complex trait variation. We discuss the relevance of studies in individual model and crop plant taxa to natural systems and to trait evolution beyond the species level. Finally, we explore potential future research directions, in light of gaps in existing data and techniques, from the standpoint of opportunities afforded by emerging technologies.

Genetic Architecture of Functional Traits

The term "genetic architecture," as used in this article, denotes the number, genomic distribution, allelic frequency, allelic effects, and interactions of genes affecting trait variation. Molecular studies of genetic architecture have become feasible over the last two decades, largely because of the revolution in DNA marker technology. In genetic mapping, markers are arranged in linkage groups, corresponding to their arrangement on chromosomes, on the basis of their cosegregation in families from controlled crosses. Associations of trait differences with particular marker alleles are used to identify chromosomal regions harboring individual genes (or multiple tightly linked genes) responsible for trait variation segregating within the cross (fig. 1). Since most of these traits vary in a continuous or quantitative fashion, these chromosomal regions are referred to as quantitative trait loci, or QTLs. In contrast to discrete Mendelian traits, quantitative traits are typically affected by variation at multiple genetic loci as well as environmental factors. As a consequence, adequate statistical power to detect and precisely locate QTLs, especially those with relatively small effects, requires mapping populations consisting of large progeny sets from controlled crosses (Beavis 1994; Falconer and Mackay 1996). QTL detection also depends on the effects of the QTL alleles that happen to be segregating within the particular family being mapped. An inbred or outcross family used for mapping will segregate for a maximum of two or four alleles, respectively, at a particular locus, while a large number of alleles with a wide range of effects on trait values may be present in the overall population. Consequently, multiple studies using crosses between different parents are likely to find different QTLs as well.

Prevalence of Major QTLs

One of the most noteworthy results of QTL studies is the fact that QTLs are in fact detected. Individual QTLs would not be found if trait variation were controlled exclusively by many genes with individually small effects. It has been quite common for mapped QTLs to explain substantial percentages of the phenotypic variance in quantitative traits. QTLs with large effects, for which the two homozygous genotypes differ by 0.5 phenotypic standard deviation or more, are commonly encountered (Falconer and Mackay 1996). QTLs of this magnitude account for substantial proportions of the within-cross phenotypic variance (table 1). Many QTL studies have been conducted using parents that have undergone divergent artificial selection, possibly increasing the frequencies of genes with large effects on the selected traits. Nevertheless, large QTLs have often been found segregating within unselected natural populations as well, as many of the examples discussed below demonstrate.

Flowering time in plants is a typical trait with respect to the

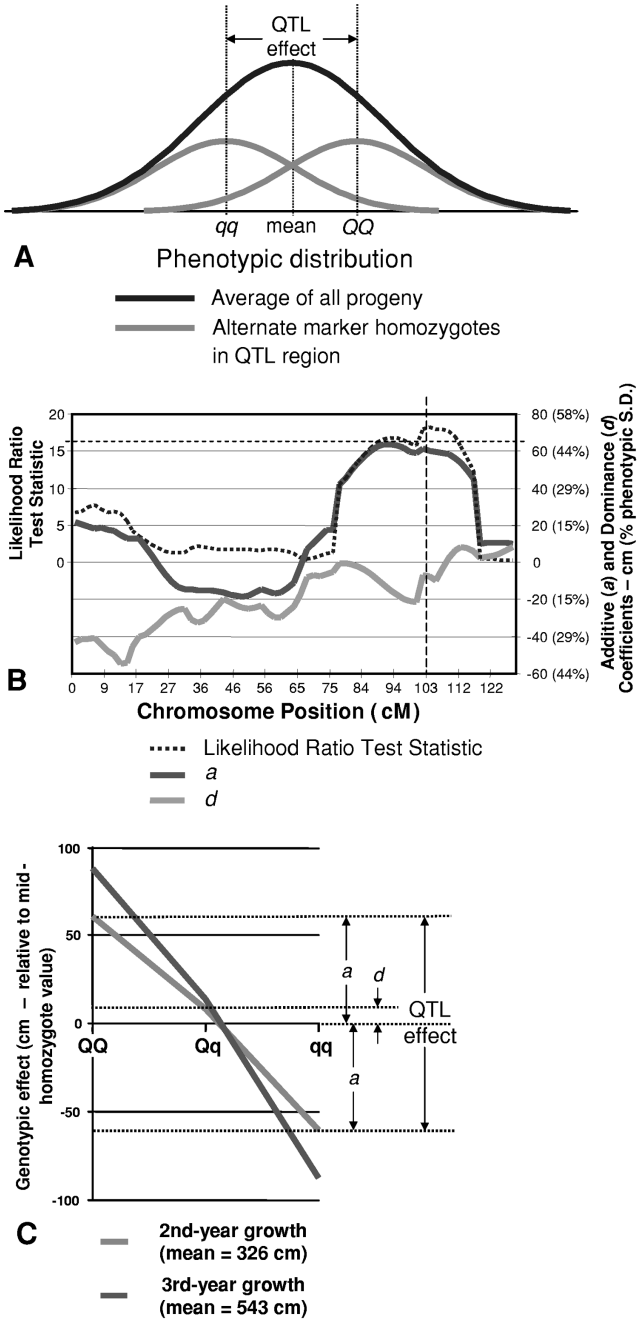

Fig. 1 Principles of QTL mapping. *A*, Phenotypic values of a quantitative trait within a full-sibling family will generally follow a normal distribution. However, alternate homozygous genotypic classes at markers in a QTL region (*QQ*, *qq*) will deviate significantly from each other in average trait values, reflecting the phenotypic effect of the QTL. *B*, QTL profile for second-year shoot growth in a loblolly pine (*Pinus taeda*) family, showing likelihood ratio test statistics and estimated additive and dominance coefficients at two centiMorgan (cM) intervals along a single chromosome. The horizontal dashed line represents the genome-wide significance threshold for the test statistic; values exceeding this level provide evidence for a QTL at or near the position of the vertical dashed line. Additive (*a*) and dominance (*d*) coefficients represent average homozygote and heterozygote trait value deviations, respectively, from the mean of the two homozygous classes. *C*, Average trait values of each genotypic class for second-year and third-year shoot growth for the QTL location shown in *B*. The QTL effect for second-year growth (2*a*) is ca. 0.88 phenotypic SD, explaining ca. 10% of the phenotypic variance. Data from Remington and O'Malley (2000).

existence and effects of QTLs. The adaptive importance of flowering phenology has long been recognized, and climatic factors, pollinator adaptations, or deleterious effects of interspecific gene flow may all function as selective mechanisms (Rathcke and Lacey 1985). Accumulated genetic differences in flowering time can result in prezygotic isolation even if they are not selectively advantageous per se. QTL mapping studies have identified locations of many of the genes that underlie natural variation in flowering time in *Arabidopsis*. A cross between the *Arabidopsis thaliana* ecotypes Landsberg *erecta* and Cape Verde Islands (L*er* and Cvi) studied under different growing conditions revealed four major QTLs for flowering time variation as well as a number of minor QTLs (Alonso-Blanco et al. 1998). Each of the major QTLs was responsible for at least 15% of phenotypic variance in at least one environment. Interestingly, the two *Arabidopsis* accessions flowered at similar times, and both ecotypes harbored early-flowering alleles that contributed to flowering time variation in the mapping populations. A recent study of inflorescence development shows that QTLs that affect flowering time also affect other aspects of *Arabidopsis* shoot architecture (Ungerer et al. 2002).

Studies of flowering time QTLs have also been undertaken in other plant species, including domesticated rice (*Oryza sativa*). Mapping experiments in crosses between *indica* and *japonica* rice cultivars identified six QTLs for heading date (Yano et al. 1997; Yamamoto et al. 1998, 2000), the first five of which explained 84% of the phenotypic variation. A separate study involving different *japonica* and *indica* rice cultivars found four major heading date QTLs, but three of these QTLs are on different chromosomes than those identified in the preceding studies (Li et al. 1995). However, both rice cultivars used in the Li et al. (1995) study were dwarf varieties, and three of the flowering time QTLs corresponded with the QTLs for plant height. As with the preceding *Arabidopsis* study, the parental lines used in these studies differed little in average heading date, both lines contributed early-flowering alleles, and some loci had very large effects.

A large number of other plant morphological and ecophysiological traits have been dissected by QTL mapping strategies, including grain yield, fruit size, floral morphology, plant height, leaf size, biomass allocation, and concentrations of enzymes and metabolites. One important ecophysiological trait that has received some attention is drought resistance. In sorghum, for example, the "stay-green" form of drought resistance results in delayed senescence and continued fruit development during postflowering drought conditions. Genetic mapping of the stay-green trait involves quantitative assessment of yield, leaf chlorophyll content, and other physiological responses under postflowering drought and has been done in sorghum (*Sorghum bicolor*; Sanchez et al. 2002) and in pearl millet (*Pennisetum typhoides*; Thomas and Howarth 2000).

Table 1

Comparison of QTL Effects and Percent of Variance Explained

	Percent phenotypic variance explained by QTL (V_Q)	
QTL effects (SD)[a]	Additive ($d = 0$)[b]	Dominant ($d = a$)[b]
0.5	3.1	4.7
1.0	12.5	18.8
1.5	28.1	42.2
2.0	50.0	75.0

[a] Difference in mean phenotype of the two homozygous classes ($2a$) in units of within-family phenotypic standard deviations.

[b] Mode of action for QTL alleles. $V_Q = 100(0.5a^2 + 0.25d^2)$.

Studies in sorghum have identified several QTLs for stay-green characters, which together explained more than half of the phenotypic variation in these qualities (Sanchez et al. 2002). Multiple studies using crosses between different parental lines appear to have identified the same set of QTLs, on the basis of map location (Tuinstra et al. 1997; Subudhi et al. 2000; Tao et al. 2000). The consistency of these QTLs among multiple sorghum crosses and in multiple environments indicates that allelic variants with large trait effects occur at relatively high frequencies at these loci among sorghum cultivars.

Finding the Genes

A key objective of QTL mapping is to identify the specific genes responsible for QTLs and the mechanisms by which they affect trait variation. Individual genes contributing to QTL effects have been identified for some developmental traits, revealing a central role for regulatory genes in complex trait variation. QTLs have been isolated using variations of the positional cloning techniques developed for isolating genes with Mendelian effects (Frary et al. 2000; Fridman et al. 2000; Johanson et al. 2000; Yano et al. 2000, 2001; El-Assal et al. 2001; Takahashi et al. 2001; Liu et al. 2002) and/or by testing candidate genes with relevant function that map to QTL regions (Doebley et al. 1995; Wang et al. 1999; Thornsberry et al. 2001). Methods for testing and verification of the isolated genes may include transformation with transgenic constructs, testing for population-level association of gene polymorphisms with phenotype differences (Thornsberry et al. 2001), or quantitative complementation testing (Doebley et al. 1995; Mackay 2001). The precise methods used depend on techniques feasible in a given organism, the nature of the gene, and its effects.

In *A. thaliana*, a number of genes that control flowering time have been identified by mutant analysis and are thus functional candidate genes for natural flowering time variation in wild populations. In a recent review, Ratcliffe and Riechmann (2002) list 38 flowering time genes that have been isolated from *Arabidopsis*, primarily, though not exclusively, by mutant analysis. These loci include the zinc-finger transcription factor gene *CONSTANS* (Putterill et al. 1995), the MADS-box transcription factor gene *FLOWERING LOCUS C* or *FLC* (Michaels and Amasino 1999; equivalent to *FLF* of Sheldon et al. 1999), and the blue-light receptor gene *CRY2* (Mockler et al. 1999). Molecular studies have shown that flowering time in plants is under complex control and is regulated by several pathways involving vernalization, perception of day length, and response to phytohormones such as gibberellin, as well as an autonomous pathway (for a recent review, see Simpson and Dean 2002). Interestingly, mutant analysis for flowering time loci has uncovered primarily regulatory and not structural loci.

Despite the large number of known candidate genes, only two loci responsible for natural variation in flowering time have thus far been isolated in *Arabidopsis*. Positional cloning based initially on QTL mapping identified the *FRIGIDA* locus as a major determinant of flowering time variation among *A. thaliana* ecotypes. The predicted protein encoded by the major trait locus *FRIGIDA* appears to be a transcriptional regulator but has no similarities to known proteins (Johanson et al. 2000). The *Arabidopsis EDI* QTL has been isolated and shown to correspond to the previously identified gene *CRY2*, which encodes a blue-light receptor protein (Mockler et al. 1999; El-Assal et al. 2001). Both *FRIGIDA* and *EDI/CRY2* have such large effects that they behave as Mendelian loci under some conditions (Alonso-Blanco et al. 1998). Three flowering time QTLs have also been isolated at the molecular level in rice. The QTL *Hd1* was found to be an orthologue of *CONSTANS* (Yano et al. 2000), and *Hd6* encodes a subunit of CK2 protein kinase, a signaling protein responsible for circadian clock mutations in *Arabidopsis* (Takahashi et al. 2001). Effects of a third rice QTL (*Hd3a*) have been isolated to an orthologue of the *Arabidopsis* gene *FLOWERING LOCUS T* (*FT*) (Yano et al. 2001), a putative ligand-binding protein involved in signal transduction (Kardailsky et al. 1999; Kobayashi et al. 1999).

At least eight individual genes responsible for quantitative (or qualitative) variation in flowering time in plants have been either isolated or strongly inferred (table 2). All of these genes are regulatory, encoding either transcription factors or proteins involved in signal transduction. Six of these genes have also been identified by mutant analysis as regulators of flowering time, although only three of the QTLs were isolated using a candidate gene approach. One of these three loci, the *FLF* QTL (Alonso-Blanco et al. 1998) in *Arabidopsis*, has not been verified to be the same locus as the *FLC/FLF* identified from mutant studies; however, both map to the same location on the top of chromosome 5, and the behavior of *FLF* QTL variants is similar to that of *FLC/FLF* mutants.

In partial contrast to developmental traits such as flowering time, both regulatory and enzyme-encoding genes appear to be important contributors to variation in metabolic traits. Mitchell-Olds and Pedersen (1998) mapped QTLs for expression levels of 10 enzymes involved in glycolysis or plant defense processes. Five of the glycolytic enzymes showed strong genetic correlations in expression levels, and a single QTL regulated levels of three of these enzymes. Other QTLs affected expression of individual enzymes, and some of these mapped to the locations of the genes encoding the enzymes. Several QTLs for glycolytic enzyme levels and glucosinolate production in *Arabidopsis* map to the locations of genes that encode the responsible enzymes (Mitchell-Olds and Pedersen 1998; Kliebenstein et al. 2001). In maize, QTLs for concentration of maysin, an important contributor to earworm resistance, have been mapped in several crosses (McMullen et al. 1998). A key

Table 2

Genes Responsible for Natural Variation in Flowering Time

Gene or locus name[a]	Species	Protein class (function)	Functional candidate	Comment	References
CONSTANS a	*Brassica nigra*	Zinc finger transcription factor (photoperiod responsive floral induction)	Yes	Actual QTL effect may be due to *CONSTANS LIKE 1*, located immediately upstream of *CONSTANS a*	Lagercrantz et al. 1996; Osterberg et al. 2002
CRYPTOCHROME 2 (*CRY2*)/*EDI*	*Arabidopsis thaliana*	Blue-light photoreceptor (photoperiod responsive floral induction)	Yes[b]	Identified as QTL, but effects are Mendelian under short day conditions	El-Assal et al. 2001
*Dwarf*8 (*D*8)	*Zea mays*	GRAS family/DELLA transcription factor (gibberellin response modulation)	Yes	Effects on flowering time inferred from association studies	Thornsberry et al. 2001
FLOWERING LOCUS C (*FLC*)/*FLF*	*A. thaliana*	MADS-box transcription factor (floral repression)	Probably	The *FLF* QTL and the characterized mutant *FLC/FLF* are likely to be the same locus, but this has not been verified	Alonso-Blanco et al. 1998; Michaels and Amasino 1999; Sheldon et al. 1999
FRIGIDA	*A. thaliana*	Novel protein family (floral repression)	No	Mendelian effects on flowering time	Johanson et al. 2000
Heading date 1 (*Hd*1)	*Oryza sativa*	Zinc finger transcription factor (photoperiod responsive floral induction	Yes[b]	Gene is orthologous to *Arabidopsis* flowering time regulatory gene *CONSTANS*	Yano et al. 2000
Heading date 3*a* (*Hd3a*)	*O. sativa*	TFL-like putative ligand binding protein (floral induction)	Yes[b]	Gene is orthologous to *Arabidopsis* flowering time regulatory gene *FT*	Yano et al. 2001
Heading date 6 (*Hd*6)	*O. sativa*	Protein kinase CK2, α subunit (circadian clock regulator)	No		Takahashi et al. 2001

Note. Genes that have been identified as flowering time genes on the basis of mutant analysis are described as functional candidates, regardless of whether a candidate gene approach was used to isolate the trait locus.

[a] Trait loci for which the responsible genes have been isolated or strongly inferred.

[b] Gene function in floral regulation was known at time of trait locus isolation, but candidate gene approach was not used to isolate gene.

maysin QTL maps to the *p1* locus, which encodes a transcription factor believed to participate in coordinate regulation of structural enzymes involved in maysin synthesis. By contrast, a tomato QTL for sugar content was isolated to an invertase-encoding gene (Fridman et al. 2000).

Regulatory Complexity of Quantitative Trait Variation

Genetic mapping can also provide key insights into the genetic mechanisms, regulatory complexity, and ecophysiological trade-offs involved in quantitative trait variation. Mapping can be used to evaluate the genetic relationships between different traits. Localization of QTLs for multiple traits to the same chromosomal region may provide at least preliminary evidence of regulation by the same set of genes (pleiotropy), genetic trade-offs among multiple traits, or the contribution of specific physiological or developmental measures to complex traits such as growth or seed production. In similar fashion, mapping of the same traits under different growing conditions or in different developmental stages can be used to gauge the complexity of trait regulation over the ecological amplitude and life span of the plant.

The results of QTL studies in *Arabidopsis* reflect the complexity of flowering time control. In their QTL study in the *A. thaliana* ecotypes L*er* and Cvi, Alonso-Blanco et al. (1998) evaluated flowering time QTLs separately in both short-day and long-day environments and both with and without vernalization in the long-day environment. Different QTLs showed different patterns of effect across environments. This design allowed analysis of the response of both alleles at each locus to vernalization and photoperiod differences. The *EDI* (*CRY2*) locus explained major proportions of the variance in flowering time in all environments but had especially large effects in short-day conditions, with the Cvi allele showing almost complete insensitivity to day length. By contrast, the effects of *FLH* were primarily the result of different levels of vernalization response, while *FLC/FLF* and *FLG* alleles showed differences in response to both photoperiod and vernalization treatments. Moreover, *FLC/FLF* and *FLG* interacted

epistatically, with significant effects on flowering time only when Cvi alleles were present at both loci. The Alonso-Blanco et al. (1998) study demonstrates the power of appropriately designed QTL experiments to illuminate the mechanisms by which different loci affect trait variation, which may subsequently be useful in identifying candidate genes.

Studies of stay-green drought resistance also indicate substantial regulatory complexity. Studies in pearl millet have identified one stay-green QTL that has phenotypic effects under both normal watering and drought conditions and is also associated with flowering time variation, while other QTLs are trait and condition specific (Thomas and Howarth 2000). A number of potential candidate genes for various drought resistance mechanisms have been proposed (Thomas and Howarth 2000; Sanchez et al. 2002), but efforts to identify the genes responsible for QTLs have been limited so far.

Plant growth is generally hypothesized to be resource driven, controlled by a large number of component processes involving resource use efficiency and resource allocation to plant defenses (Herms and Mattson 1992; Ackerly et al. 2000). Plant growth per se is an important adaptive trait, as it is a key determinant of competitiveness, but may also have negative functional correlations with production of defense chemicals (Herms and Mattson 1992; but see Lerdau et al. 1994), flowering (Geber 1990), and tolerance of environmental extremes (Ma 1987; Rehfeldt 1992; Schmidtling 1994). If these models are correct, plant growth should be controlled by many genes with individually small effects, and any QTLs large enough to be detected should be associated with component traits. Similar arguments can be made for reproductive output, manifest as yield in grain crops (Ishimaru et al. 2001). The QTL mapping data available so far, however, do not support these predictions. Ishimaru et al. (2001) developed a "function map" of rice QTLs for a variety of agronomic, physiological, and morphological traits onto a cross between *japonica* and *indica* varieties using a common set of genetic markers. QTLs for grain yield and photosynthetic efficiency did not map to the same locations as QTLs for the presumed developmental and physiological measures. QTLs for yield did not overlap with QTLs for flag leaf chlorophyll content, occupied space, or space per stem, contrary to expectations based on phenotypic correlations among traits. Similarly, QTLs for photosynthetic efficiency did not correspond to those for Rubisco : chlorophyll or chlorophyll *a* : *b* ratios or for measures of intercellular CO_2 concentration. Moreover, neither grain yield nor photosynthetic efficiency QTLs corresponded to the locations of genes encoding several enzymes important in carbon metabolism (*rbcS*, cystolic and plastidic *FBPase*, *R-enzyme*, and *sucrose synthase*).

Studies of shoot growth in trees over multiple years have identified QTLs that explain large percentages of the phenotypic variation, indicating that mechanisms of growth regulation may be more direct than has been supposed. Bradshaw and Stettler (1995) studied a number of traits related to growth and development in an interspecific cross between *Populus trichocarpa* and *Populus deltoides* over two growing seasons. Nearly half of the variation in stem volume after 2 yr was explained by two QTLs. Several QTLs affecting various aspects of radial, shoot, and leaf growth were clustered at a single location, indicating that a single growth regulatory locus may be responsible for a number of evolved interspecific differences in growth patterns. However, there were a number of year-to-year differences in the specific QTLs that were detected, which may indicate a complex regulatory network similar to that of flowering time. Similar results have been obtained in eucalyptus (Verhaegen et al. 1997) and loblolly pine (Kaya et al. 1999). In a study of plant height in rice, strong QTL-by-environment and QTL-by-QTL (epistatic) interactions were found, indicating the presence of interacting growth regulatory mechanisms that respond to a variety of environmental cues (Cao et al. 2001).

Limitations of QTL Studies

Several caveats must be borne in mind when interpreting the results of QTL studies. A basic but often overlooked consideration is that QTLs will only be detected when alleles with significantly different trait effects are segregating in the mapped cross or pedigree. Consequently, failure to detect a QTL near the location of a candidate gene in a particular cross does not necessarily rule out involvement of the gene in trait variation at the population level (Ishimaru et al. 2001). In the same manner, the failure to detect common QTLs for genetically correlated traits does not rule out the existence of shared regulatory genes but may only indicate that any such loci lack meaningful polymorphisms in the study families. It is also difficult to determine whether a detected QTL represents large effects of a single gene or more modest effects of multiple linked loci. In cases where multiple loci are linked in repulsion (i.e., alleles at two loci on the same parental chromosome have opposite effects on the trait value), major QTLs may go undetected. Similarly, QTLs affecting multiple traits may be the result of multiple linked loci rather than pleiotropy.

Statistical issues can also lead to bias in QTL experiments. Genome-wide QTL detection studies involve a large number of separate tests of different chromosomal regions, so very stringent statistical criteria are required to minimize the occurrence of false positives. Consequently, a sample size of at least several hundred individuals is usually required for adequate power to reliably detect any but the largest QTLs (Beavis 1994). Many published QTL studies lack these numbers, so many QTLs have probably gone undetected, and the effects of QTLs that are detected may be overestimated. Failure to detect the same QTLs in repeated measurements of a trait, as in the tree growth studies discussed previously, has been commonly interpreted as evidence of stage or environmental specificity, but such inferences should be made with caution. In QTL studies of floral morphology in an interspecific cross of the monkeyflower taxa *Mimulus lewisii* and *Mimulus cardinalis*, Bradshaw et al. (1995, 1998) detected more than double the number of QTLs for a variety of traits in a study with 465 mapped progeny than in an earlier study with only 93 individuals. All but one of the 12 QTLs identified in the first study were found in the second, but the estimated magnitudes of all of these QTLs were smaller in the second study. Another form of ascertainment bias may stem from the nature of scientific literature itself; studies that detect significant QTLs may be more likely to be published than those with negative results.

Finally, gene interactions (epistasis) can affect the detection of QTLs and their apparent magnitude. The power to detect

specific epistatic interactions is especially limited by multiple testing considerations in QTL experiments because the number of possible two-way interaction terms increases approximately in proportion to the square of the number of main effects. Doebley et al. (1995) found that the effects of QTLs for plant architecture differences between maize and teosinte varied depending on the genetic background in which they were evaluated. More recently, Lauter and Doebley (2002) also found genetic variation for some of the same plant architecture QTLs within teosinte when hybrids between two teosinte subspecies were testcrossed to maize. QTL effects for ear disarticulation, number of ear internode ranks, and percentage of internodes with pedicellate spikelets were detected between the teosinte subspecies in the maize background, even though the traits are invariant within and between the two teosinte subspecies. These findings indicate that observed effects of major trait loci may depend in part on substitutions that occurred at other loci as well.

The net result of all these factors is that the aggregate genetic basis of quantitative trait variation is likely to involve more loci with smaller effects than those reported in initial QTL studies. Nevertheless, there is very little indication that the basic conclusions of initial studies reporting detection of major QTLs are being invalidated by further research. In spite of its potential shortcomings, QTL analysis has been and is likely to remain a critical tool for genetic analysis.

How Applicable Are the Findings?

Perhaps a more critical issue from the standpoint of this discussion is whether the results from plant QTL and functional studies are applicable to evolution in natural systems. Studies in crop plants detect trait differences that in many cases are the result of artificial selection, the nature and intensity of which may be very different from those of natural selection in the wild. Highly selected crop plants may retain only small proportions of the genetic variation present in their wild ancestors, although this varies somewhat by species (Tanksley and McCouch 1997; Eyre-Walker et al. 1998). Natural populations of the self-fertilizing *A. thaliana* may also be atypical in that fixation of deleterious alleles might be much more prevalent in selfing plants than in outcrossing taxa (Bustamante et al. 2002). Moreover, we need to ask whether the kinds of polymorphisms segregating within natural or artificial populations are relevant to the evolutionary differences that arise between species. While differences between species must have their origins in variation that arises within populations (Purugganan 2000), it has also been argued that the evolutionary dynamics of species divergences are fundamentally different than those of intraspecific variation (Gould 1980).

Perhaps the strongest conclusion that can be drawn so far is that individual genes with relatively large effects on trait variation are probably important in evolution. Identification of QTLs with moderate to large effects has been a nearly universal occurrence in published QTL studies (Falconer and Mackay 1996), and this seems to be true in plants. Although many of the studies we have discussed above have been done in crop plants, the studies in intraspecific selections from natural populations (Alonso-Blanco et al. 1998; Mitchell-Olds and Pedersen 1998) and interspecific hybrids (Bradshaw and Stettler 1995; Bradshaw et al. 1995, 1998) have yielded similar results. Crosses between natural accessions with very similar phenotypes have been found to harbor QTL variants with effects large enough to behave as Mendelian loci (El-Assal et al. 2001). Thus, it appears that limited numbers of mutations are likely to explain a large share of both natural intraspecific variation and adaptively important evolutionary differences between taxa, at variance with the micromutationist perspective. Recent theoretical studies by Orr (1998) analyzing the predicted distribution of adaptive mutational effects provide further reinforcement to these empirical results. One implication of these theoretical and experimental results is that major phenotypic differences between differentiating populations could arise relatively rapidly from natural selection on major QTLs.

The prominent role of regulatory loci in trait variation is also becoming clear, although data at the interspecific level are limited. The QTLs that have been isolated for plant morphological and life-history traits have all been genes coding for transcriptional regulators or signaling proteins. These findings are consistent with the results of *Drosophila* bristle number studies, in which the major QTLs have been associated with polymorphisms in neurogenic regulatory loci (Mackay and Langley 1990; Lai et al. 1994; Long et al. 1998, 2000). For metabolic traits, however, genes encoding relevant enzymes appear more likely to be involved.

Another concept emerging from the preceding studies is that variation in complex traits may be largely the result of variation in genes that directly regulate the traits themselves, rather than the secondary result of regulation of correlated processes. Most variation in flowering time is controlled by genes that affect how plants perceive and respond to various environmental and developmental cues involved in the transition to flowering. Whether comparable models will apply for other complex traits is less certain. The lack of correlation between QTL locations for grain yield and its presumed physiological components in rice function mapping (Ishimaru et al. 2001) indicates that developmental rather than physiological mechanisms may be primarily responsible for genetic variation in fecundity in grasses. The identification of major QTLs for tree growth and plant height and the apparent importance of epistatic and environmental interactions in growth variation (Bradshaw and Stettler 1995; Cao et al. 2001) seem to indicate that regulation of plant growth has many similarities to that of flowering time. Genes whose primary function is direct regulation of growth have been found to be responsible for agronomically important plant size variants in cereal crops (Peng et al. 1999; Sasaki et al. 2002), but a role in natural interspecific divergences has yet to be demonstrated. If the emerging view of plant growth regulation is correct, the nature of evolutionary trade-offs between growth and other processes may be more of a two-way street than has been supposed. Growth, fecundity, stress resistance, and defense processes may all be directly controlled by genes undergoing selection pressures, with relatively minor pleiotropic effects on other traits acting as evolutionary constraints.

Contrary to the predictions of Doebley and Lukens (1998), the flowering time QTLs that have been isolated so far appear to be primarily the result of coding region mutations that give rise to altered or truncated regulatory proteins, rather than changes in their expression patterns. The *Hd6* and multiple

Hd1 variants in rice involve mutations leading to altered proteins (Yamamoto et al. 2000; Yano et al. 2000), as do the *EDI* (*CRY2*) and the Mendelian-segregating *FRIGIDA* variants in *Arabidopsis* (Johanson et al. 2000; El-Assal et al. 2001). However, this may reflect the idiosyncrasies of artificial selection in crop domestication and of inbreeding in *A. thaliana*. The effects of *teosinte branched 1* (*tb1*) and *c1* in maize and teosinte, which are responsible for major variation in plant architecture and anthocyanin pigmentation differences, respectively, are related to changes in gene regulation rather than coding region changes (Hanson et al. 1996; Wang et al. 1999). Domestication in maize does not appear to have been accompanied by severe genetic bottlenecks (Eyre-Walker et al. 1998; Wang et al. 1999; Remington et al. 2001), and it is possible that fewer mutations involving wholesale alterations or losses of protein function became fixed in maize as a result.

While genes with large effects have probably been important in trait evolution, the extent to which the same genes have contributed to parallel evolutionary changes in multiple lineages is unknown. It appears possible that some genes or gene families will turn out to have broad importance in the evolution of adaptive variability. The *Hd1* QTL in rice was found to be a homologue of *CONSTANS* (*CO*), which had already been identified from mutant studies as an important photoperiod regulator in *Arabidopsis* and is thus a functional candidate gene for natural variation in flowering time (Yano et al. 2000). Recently, a major flowering time QTL in *Brassica nigra* was found to be strongly associated with nucleotide variation in a homologue to *CONSTANS LIKE 1* (*COL1*), which is located only 3.5 kb upstream from the *B. nigra CONSTANS* orthologue *COa* and in possible *COa* regulatory sequences between *COL* and *COa* (Lagercrantz et al. 1996; Osterberg et al. 2002). Whether the phenotypically important polymorphism(s) turn out to reside in the *COL* coding region or *COa* regulatory regions, it is highly noteworthy that *CONSTANS*-like genes have been implicated as flowering time QTLs in two different species, given the limited number of flowering time QTLs that have been isolated.

However, the results of other studies of developmentally important gene families call for caution in extrapolating from domesticated to wild plants. The maize *tb1* locus, originally identified as a maize plant architecture mutant, was found to be responsible for important plant architecture differences between maize and teosinte (Doebley et al. 1995). However, *tb1* variation shows no association with phenotypic differences within maize (Thornsberry et al. 2001), and analyses of *tb1* sequences within the Andropogoneae tribe of grasses show no evidence of natural selection (Lukens and Doebley 2001). Orthologues of the gibberellin response regulators *GAI* and *RGA*, originally identified from gibberellin response mutants in *Arabidopsis*, have been found to be responsible for the dwarf phenotypes of important "green revolution" wheat varieties (Peng et al. 1999) and to be associated with variation in flowering time in maize (Thornsberry et al. 2001). Molecular evolutionary analyses of *GAI/RGA* homologous sequences from the Hawaiian silversword alliance, however, showed no evidence of selection on the coding regions in this plant lineage that has undergone rapid morphological diversification (Remington and Purugganan 2002).

A serious shortcoming of QTL studies in natural populations to date is the lack of demonstration that identified QTLs actually have adaptive significance. Traits such as flowering time are undoubtedly important for adaptation in many circumstances (Johanson et al. 2000; El-Assal et al. 2001), but it does not necessarily follow that all occurrences of genetic variation in flowering time are adaptive. Ecological research demonstrating adaptive significance of QTLs in particular instances of phenotypic differentiation is often lacking in QTL studies. A notable exception is the study of Schemske and Bradshaw (1999), in which pollinator preference was evaluated in F_2 hybrids between the bee-pollinated *M. lewisii* and its hummingbird-pollinated sister species *M. cardinalis*. Floral morphology and pigmentation, for which QTLs had been mapped previously in this cross (Bradshaw et al. 1995, 1998), were shown to contribute significantly to differences in pollinator visitation.

Into the Wild

Model plants such as *Arabidopsis* and rice offer numerous advantages for genetic architecture studies, which will generally be more difficult in wild plants. Fine structure mapping and cloning of QTLs are feasible in these plants because their genomes are relatively small, and short generation times allow the required advanced-generation crosses to be produced in a relatively short time. In addition, critical resources for positional cloning such as high-density genetic maps and large-insert genomic libraries (e.g., bacterial artificial chromosome, or BAC, libraries) are already available in these species. The completed genome sequence in *A. thaliana* and ongoing genomic sequencing in rice have provided further resources for identifying and locating candidate genes in chromosomal regions of interest (Lukowitz et al. 2000). Few, if any, of these resources are available in wild plants other than *A. thaliana*. Moreover, other issues such as long generation times, long life spans over which developmental traits must be measured, large genomes, self-incompatibility, and high levels of inbreeding depression will complicate detailed genetic studies in many taxa (Rieseberg and Buerkle 2002).

Nevertheless, the evolution of many adaptively important functional traits cannot be studied in the limited number of herbaceous annual model plant systems. Some of the richest examples of evolution in morphological and ecophysiological traits with recognized adaptive function (Robichaux et al. 1990; Kim et al. 1996) are in wild nonmodel plant lineages, which will need to be studied directly if the genetic basis for their trait differences is to be understood. Fortunately, the wealth of genetic resources and data generated from model plants should greatly facilitate evolutionary studies in other plant lineages. Functionally relevant genes identified in model plants are likely to have homologues with similar functions in other plant species. These homologues can be isolated from wild taxa and evaluated for evidence of selection using methods from molecular evolution and population genetics (Barrier et al. 2001; Lukens and Doebley 2001; Remington and Purugganan 2002). Polymorphisms in prospective candidate genes can also be developed as genetic markers and located on genetic maps, where they can be evaluated for co-location with QTLs.

The degree of gene sequence similarity and colinearity on chromosomal segments can be used to help identify and isolate

trait loci in taxa closely related to model plants. The close relationship of the genus *Brassica* to *A. thaliana* was exploited in identifying a *CO* homologue as a candidate gene for a major flowering time QTL in *B. nigra* (Lagercrantz et al. 1996) and subsequent identification of DNA sequence polymorphisms associated with the trait variation in the *B. nigra COa/COL1* region (Osterberg et al. 2002). Genome colinearity is likely to be useful for comparative mapping even across much larger evolutionary divergences. A sequenced 105-kb BAC fragment from tomato showed substantial conservation of gene content and order with three different chromosomal regions in *Arabidopsis*, even though the respective taxa are in the rosid and asterid lineages that diverged ca. 125 million yr ago (Ku et al. 2000). Extensive genome colinearity has been found in the grasses as well, both at large and small scales (Chen et al. 1997; Gale and Devos 1998).

Construction of genetic linkage maps in previously unmapped plants is greatly simplified with marker techniques such as amplified fragment length polymorphisms (AFLP) because many markers can be generated and scored in a short period of time (Vos et al. 1995; Myburg et al. 2001). Using AFLP markers with or without additional marker types, *de novo* construction of genetic maps with thorough genome coverage has been feasible (Remington et al. 1999; Fishman et al. 2001). Linkage and QTL mapping strategies have been developed for outbred pedigrees in plants that are self-incompatible or have high levels of inbreeding depression (Sewell et al. 1999; Sillanpaa and Arjas 1999) or that have heterozygous parents (Grattapaglia and Sederoff 1994; Grattapaglia et al. 1996). As a consequence, genetic mapping should be feasible in a wide variety of plant taxa, provided they can be crossed to produce mapping populations of sufficient size. Hybrid breakdown resulting from chromosomal rearrangements and gametic or postzygotic incompatibilities may provide an impediment to mapping in interspecific hybrids because of the difficulty of obtaining progeny, suppressed recombination, and distorted segregation ratios.

It will be difficult to produce the advanced-generation backcrosses necessary for fine-structure mapping and cloning of QTLs in many wild taxa, especially those that have long generation times or are poorly suited for inbreeding. Where candidate genes can be identified, population-level testing for associations between sequence polymorphisms and trait values will generally be feasible. Association methods were originally developed for human gene discovery, where experimental mapping populations and transgenic constructs cannot be used. The resolving power of association methods results from the much larger number of recombination events that have occurred in the lineage of an entire population, compared with a controlled pedigree of only a few generations at most (Weir 1996). Consequently, the sizes of chromosomal regions in which genetic polymorphisms are in linkage disequilibrium (LD) with mutations responsible for trait differences are likely to be very small. The actual sizes of regions in LD, however, depend on such factors as recombination rates, historic effective population size, ages of the respective mutations, selection on individual regions, and stochastic variation (Nordborg and Tavare 2002). Factors not related to linkage, such as population structure, can also result in LD, but methods to identify and control for the effects of population structure have been developed (Pritchard et al. 2000*a*, 2000*b*; Thornsberry et al. 2001). Association methods have recently been used to evaluate associations between flowering time and polymorphisms at the *dwarf8* (*d8*) locus in maize (Thornsberry et al. 2001) and in the *COa/COL1* region in *B. nigra* (Osterberg et al. 2002). In addition, population genetic methods are also proving useful for evaluating whether selection or neutral evolutionary processes have been involved in generating polymorphism or divergence in candidate genes, at least in crop plants (Wang et al. 1999; Vigouroux et al. 2002).

Association methods will have critical limitations for testing of candidate genes in chromosomal regions identified by QTL mapping. Typical QTL mapping experiments using first-generation backcross, F_2, or recombinant inbred populations will seldom localize QTL effects to regions smaller than 10–20 centiMorgans (cM). A recent study of inflorescence development QTLs in *Arabidopsis* recombinant inbred lines determined that 375–783 genes lay within the 10–21 cM confidence intervals for QTL location (Ungerer et al. 2002). Identifying the responsible genes within such regions by candidate gene methods alone will be a daunting task, especially considering that previously uncharacterized genes may be responsible. For example, the recent cloning of the *FRIGIDA* locus with major effects on *Arabidopsis* flowering time revealed a gene with no similarity to any known gene families (Johanson et al. 2000), let alone identity with known flowering time mutants.

The Road Ahead

Data and Technical Gaps

Molecular studies of gene function and genetic architecture are challenging the prevailing views of genetic control of functional trait evolution. Individual loci with large quantitative effects frequently explain most of the genetic variation in study populations, in contrast to the micromutationist perspective of the neo-Darwinian synthesis. Moreover, this seems to be equally true for "complex" morphological and developmental traits and for specific metabolic traits. Contrary to ecophysiological models of complex trait regulation, variation in plant growth and seed yield show evidence of direct genetic regulation, independent of loci controlling hypothesized component processes. However, a number of conceptual gaps remain. Orr and Coyne's (1992) challenge to evolutionary biologists to expand the use of molecular marker techniques to study trait evolution in natural populations, issued a decade ago, has only been taken up by a handful of researchers. Only a small fraction of the research on plant genetic architecture and gene functional characterization has been conducted in wild plant populations, and even less has been done at the interspecific level relevant to speciation processes. Only one relatively complex trait (flowering time) is beginning to be well characterized in terms of both its genetic regulation and the architecture of its genetic variation in multiple taxa. There is a need to extend both the taxonomic breadth and the depth of trait analysis encompassed by studies of functional genetic variation.

As methods of genetic analysis become more widely applied to studies of natural populations, greater attention will need to be given to the ecological relevance of the traits being stud-

ied. The quantitative traits chosen for detailed study in plants are typically those with agricultural importance, and their adaptive importance in natural environments is often assumed but seldom rigorously tested. Interdisciplinary investigations using a combination of ecological, genetic, and physiological expertise are much more likely to generate useful insights into functional trait evolution than are separate studies conducted within the confines of individual disciplines.

The model genetic system represented by *Arabidopsis thaliana* and its outcrossing relatives (e.g., *Arabidopsis lyrata* and *Brassica* spp.) is gaining value for evolutionary studies because of the advantages offered by the extensive genetic resources and information base (Lukowitz et al. 2000). There is some danger, however, that kinds of trait variation not represented in well-characterized members of the Brassicaceae will be largely ignored. The canonical plant life history represented by *A. thaliana* and most of its close relatives entails an initial vegetative rosette stage, followed by a single transition to a reproductive stage in which inflorescence shoots bolt and flower. Identifying the genetic mechanisms regulating this program and its variability, however, may have limited value for understanding adaptive evolution among taxa with different morphologies and life histories. For example, in the not-too-distantly-related Myrtaceae, the "vegetative rosette" of *Eucalyptus* spp. can exceed 80 m in length, and myriads of separate vegetative-to-inflorescence transitions occur on lateral shoots over a centuries-long life span.

Genetic studies in plant lineages that have undergone recent adaptive radiation in ecophysiological and morphological traits, such as the Hawaiian silversword alliance (Robichaux et al. 1990) and the Macaronesian Sonchus alliance (Kim et al. 1996), would be especially valuable for gaining a greater understanding of trait evolution. Appropriate study systems would have to consist of species in which fertile hybrids can be generated, in order to allow the use of genetic approaches. Effective use of adaptive radiations as genetic systems would require identification of adaptively important traits, genetic marker development, establishment of appropriate crosses for mapping, and generation of cDNA libraries from which expressed sequence tags (ESTs) can be sequenced for gene discovery. In addition, construction of large-insert genomic libraries from one or more species and development of plant transformation technologies would be desirable. Selecting the most suitable lineages for such "adaptive study systems" would require consideration of both the range of phenotypic diversity encompassed by interfertile taxa and their tractability for application of a variety of genetic approaches. These systems would probably not be used for extensive gene functional characterization or genome sequencing, which can be pursued most effectively in existing model plants. However, the mere ability to do detailed mapping of genomic regions contributing to species differences in functional traits, to map candidate genes, and to test for functional involvement of candidate genes in functional trait variation using association methods would be of great value.

An area that especially warrants more detailed study is the extent and molecular basis of evolutionary genetic correlations between plant growth measures, such as height and internode elongation, and ecophysiological traits such as efficiency of photosynthesis and resource use, carbon and nutrient allocation, and production of defense chemicals. Each of these traits is likely to have broad importance in adaptive evolution, and the nature of evolutionary trade-offs among these traits has been the subject of long-standing theoretical and experimental interest (Herms and Mattson 1992; Ackerly et al. 2000). The "function mapping" approach of Ishimaru et al. (2001) may provide a useful model for future studies, but it will be important to identify taxa and populations with genetic variation relevant to evolution in natural environments. Ideal study systems would involve plants with shoot growth in vegetative as well as reproductive stages, so effects of selection primarily based on flowering time could be factored out. The Hawaiian silversword alliance, for example, includes pairs of interfertile sister taxa with large differences in size, vegetative growth rates, and habitat requirements that might be especially useful for mapping studies (Robichaux et al. 1990; D. L. Remington, unpublished data), although study designs that accommodate their woody perennial life history will be required. Such mapping studies would be the first step in identifying the actual genes, and therefore the genetic mechanisms, involved in growth and ecophysiological variation in wild populations.

Detailed QTL and functional studies of the same traits in multiple lineages would shed light on the diversity of mechanisms by which trait variation can evolve. While major genes are clearly involved in evolutionary processes, we do not know how often the same genes contribute significantly to trait variation within different plant lineages. Current information is limited and largely anecdotal. The involvement of *CONSTANS*-like genes in flowering time variation in both rice and *Brassica* indicates that at least some gene families may play key roles in overall plant diversification. QTLs for quantitative traits in several cereal grains are located in corresponding chromosomal regions, implying that the same genes may be involved (Paterson et al. 1995). However, selection for stay-green traits in different cereal grasses appears to involve different genetic mechanisms (Thomas and Howarth 2000). Specially designed comparative studies will be needed to determine whether some genes have had key roles in functional trait evolution throughout the plant kingdom.

New and Emerging Tools

The "end game" of identifying and verifying genes responsible for QTLs is likely to be challenging for nonmodel wild plant study systems. Association methods could become an important tool for testing genes identified as candidates on the basis of function and map location. It has been suggested that association methods could be used for genome-wide screening for trait loci if enough well-distributed polymorphisms were available (Risch and Merikangas 1996). Screening across more limited chromosomal regions of interest has already been used to identify loci that may have undergone recent selection in *Drosophila melanogaster* (Harr et al. 2002). While this approach is not likely to be feasible in the near future for nonmodel plants, it may be possible to do exhaustive screening in short regions around QTLs, provided that dense genetic maps and contigs of overlapping BAC clones could be constructed.

The use of natural hybrid zones as genetic mapping populations has been suggested as an alternative to establishing populations from controlled crosses (Rieseberg et al. 2000).

Rieseberg and Buerkle (2002) evaluated the feasibility of QTL mapping in hybrid zones using *Helianthus annuus* and *Helianthus petiolaris* hybrids for which standard linkage maps had already been constructed. Constructing linkage and QTL maps with the hybrid populations presented a number of difficulties but may be feasible in some circumstances with appropriate selection of markers and in relatively young hybrid zones. If linkage maps with markers that differentiate the parental species have already been constructed, subsequent fine-structure QTL mapping will be facilitated. In older hybrid zones, evaluation of marker-trait associations will be more similar to association testing than to QTL mapping. Older hybrid zones may provide unique opportunities for association studies to identify loci responsible for traits that differ between species. The genome-wide distribution of fixed genetic differences between species would make association studies completely unreliable in samples from the parental species, but LD resulting from species admixture will be effectively eliminated within three to five generations of random mating among hybrids.

Recently developed genomic and proteomic techniques allow expression levels of thousands of genes to be evaluated in single experiments, further breaking down the barriers between the functional and evolutionary levels of inquiry. Microarrays can be used to identify genes that are transcribed at different levels in contrasting genotypes, tissues, environments, or developmental stages. Genomic or cDNA sequences from thousands of expressed genes are spotted on a glass slide or membrane and probed with dye-labeled cDNA representing the contrasting conditions (Lashkari et al. 1997). Genes showing different transcript levels are likely to be involved in regulatory pathways affecting the trait or response being evaluated. A potential evolutionary genetics application of microarray technology would be to evaluate transcript levels in functionally appropriate tissues from bulked samples of individuals with contrasting marker genotypes at relevant QTLs. Genes showing different levels of expression in the respective QTL classes would be likely to be involved in trait regulation. Moreover, differentially expressed genes that map to the same location as the QTL would become strong candidates for the QTL. A recent application of this approach (although not in a plant) was to identify a positional candidate for a QTL for Marek's disease resistance in chickens (Liu et al. 2001). This method would only be useful for identifying the actual QTL in cases where the functional mechanism is a change in the responsible gene's expression level. However, identifying QTL effects on expression of a number of "downstream" genes may provide insights on the regulatory networks involved in the trait variation, yielding clues to the identity of the actual QTL. Potentially less expensive alternatives to microarray techniques, such as differential display (Bauer et al. 1993) and cDNA-AFLP (Bachem et al. 1996), may make gene expression profiling more feasible in wild taxa in which funds and molecular resources are limiting. Similar approaches using two-dimensional protein electrophoresis can be used to study gene expression differences at the protein level (Consoli et al. 2002).

Finally, genome sequencing and construction of genomic and expressed gene libraries in model plants are providing phenomenal insights on the evolution of entire genomes. The roles of polyploidy and genome duplication in plant evolution are poorly understood, but duplicated genes can evolve new or reallocated functions with the potential to generate new phenotypes (Wendel 2000). Alternatively, the complementary loss of function of duplicated genes in sister lineages can contribute to speciation by generating hybrid incompatibilities (Lynch and Force 2000). Much of the genome history represented by the sequential duplication of chromosomal regions, or even the entire genome, in *Arabidopsis* (Vision et al. 2000) will be common to all flowering plants. Consequently, insights gained from genomic sequencing will add greatly to our understanding of the overall role of genomic phenomena in plant evolution.

Summary

The list of published studies describing QTL mapping of functional traits is huge and growing rapidly, as is the amount of information accumulating on gene function related to important traits. We have by necessity focused on a small subset of studies to illustrate the main themes that are emerging on the genetics of functional trait evolution in plants. Undoubtedly, we could have selected many other studies that exemplify the same points. As with all scientific concepts, some of the new ideas of phenotypic evolution that are emerging from recent studies are likely to be substantially incorrect and will eventually be replaced with more realistic understandings. Nevertheless, the rapid expansion of molecular tools and the resulting information are bringing us closer than ever before to understanding how the phenomenal phenotypic diversity of the angiosperms came into being.

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