Int. J. Dev. Biol. 49: 717-732 (2005)
doi: 10.1387/ijdb.051994ca

From phenotypic to molecular polymorphisms involved in naturally occurring variation of plant development

CARLOS ALONSO-BLANCO*,[1], BELÉN MENDEZ-VIGO[1] and MAARTEN KOORNNEEF[2,3]

[1]Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología, CSIC, Madrid, Spain, [2]Laboratory of Genetics, Wageningen University, The Netherlands and [3]Max Planck Institute for Plant Breeding Research, Cologne, Germany

ABSTRACT An enormous amount of naturally occurring genetic variation affecting development is found within wild and domesticated plant species. This diversity is presumably involved in plant adaptation to different natural environments or in human preferences. In addition, such intraspecific variation provides the basis for the evolution of plant development at larger evolutionary scales. Natural phenotypic differences are now amenable to genetic dissection up to the identification of causal DNA polymorphisms. Here we describe 30 genes and their functional nucleotide polymorphisms currently found as underlying allelic variation accounting for plant intraspecific developmental diversity. These studies provide molecular and cellular mechanisms that determine natural variation for quantitative and qualitative traits such as: fruit and seed morphology, colour and composition; flowering time; seedling emergence; plant architecture and inflorescence or flower morphology. Besides, analyses of flowering time variation within several distant species allow molecular comparisons between species, which are detecting homologous genes with partly different functions and unrelated genes with analogous functions. Thus, considerable gene function differences are being revealed also among species. Inspection of a catalogue of intraspecific nucleotide functional polymorphisms shows that transcriptional regulators are the main class of genes involved. Furthermore, barely more than half of the polymorphisms described are located in coding regions and affect protein structure, while the rest are regulatory changes altering gene expression. These limited analyses of intraspecific developmental variation support Doebley and Lukens´s proposition (1998) that modifications in *cis* -regulatory regions of transcriptional regulators represent a predominant mode for the evolution of novel forms, but await more detailed studies in wild plant species.

KEY WORDS: *evolutionary developmental biology (evo-devo), intraspecific (within-species) variation, quantitative trait locus (QTL), functional polymorphism, adaptation, domestication*

Naturally occurring intraspecific variation: a genetic resource for the study of adaptation and evolution of plant development

Plant diversity has fascinated mankind throughout history, primarily due to the tremendous variation that exists in nature for morphological and other developmental traits. The fitness effects of such naturally occurring variation present among species (interspecific) have driven plant (macro)evolution by natural selection, this developmental diversity being the basis of plant taxonomy and phylogeny (Cronk, 2001). In addition, comparable developmental variation exists within many species (intraspecific), which likely reflects adaptations to different natural environments and it is the origin of plant species differentiation (Linhart and Grant, 1996). Humans have used this intraspecific variation for the domestication and genetic improvement of more than 100 plant species (Diamond, 2002) by applying directional selection on multiple aspects of plant development. In this way, varieties adapted to agricultural environments and with increased yield have been generated for many cultivated plant species of interest as human food or ornament.

A major challenge of current biology is to understand the genetic basis and molecular mechanisms of all this naturally occurring developmental variation at the nucleotide, protein and cellular level. This analysis has begun only recently thanks to the availability of whole genome sequences, which allow the development of genomic tools aimed to identify gene functions and the mechanistic basis of phenotypes in model plant species like

Abbreviations used in this paper: QTL, quantitative trait locus.

***Address correspondence to:** Dr. Carlos Alonso-Blanco. Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología (CNB-CSIC) Darwin 3, Cantoblanco, 28049 Madrid, Spain. Fax: +34-585-4506. e-mail: calonso@cnb.uam.es

0214-6282/2005/$25.00

Printed in Spain
www.intjdevbiol.com

Fig. 1. Developmental variation in the domesticated species *Phaseolus vulgaris* L. *Cultivated varieties of common bean differ quantitatively or qualitatively for the following traits: growth habit, which ranges from indeterminate climber to determinate postrate* **(A)***; morphology of leaves, leaflets and petioles* **(B)***; flower colour and pedicel length* **(C)***; size, shape and colour of fruits* **(D)***; size, shape and colour of seeds* **(E)***. Plants were grown together and samples were collected from comparable nodes, at the same age; pictures in each panel are at the same magnification.*

Arabidopsis thaliana (*Arabidopsis* hereafter) and rice. Gene functional studies are providing candidate genes that might contribute to interspecific diversification and evolution of plant development (so-called "evo-devo"), which are investigated in broad comparative phylogenetic developmental studies (reviewed in Cronk, 2001; Shepard and Purugganan, 2002; Irish and Benfey, 2004; Kellogg, 2004). In contrast, intraspecific variation can be currently dissected by genetic analysis, which identify the actual genes and nucleotide polymorphisms causing the observed diversity. Thus, the analysis of natural intraspecific variation is delivering: first, the genes involved in microevolution of developmental processes and in plant adaptation through developmental modifications. Secondly, the precise alleles and functional nucleotide polymorphisms causing natural variation, which allow the elucidation of the molecular mechanisms changing and differentiating gene functions during evolution within species. This information extends our current concept of gene function, which is mainly based on the characterization of a single natural "wild type" allele of a species. Studying the effects of different natural alleles in a context depending on genotype, environment and evolutionary temporal scale provides a more realistic and dynamic perspective of gene function (or effect) (Tonsor *et al.*, 2005).

In this review we summarize current molecular studies of naturally occurring developmental variation within plant species, or among closely related species amenable to genetic analysis by interbreeding. We define natural variation in a broad sense as phenotypic variation caused by spontaneously arisen genetic polymorphisms that are maintained either by natural or human selection. Thus, we include variation: within wild species; within domesticated species; and between domesticated species and their wild relatives and ancestors. We briefly report prevailing methodologies to dissect genetic intraspecific variation and describe the first genes and nucleotide polymorphisms that have been recently discovered underlying developmental variation in wild and domesticated species.

Natural intraspecific diversity of plant development

Variation in cells, organs and structures of the plant body.

Plant developmental traits, defined merely as the morphological and colour features that are determined by cell division, expansion and differentiation throughout the plant life cycle, include an enormous set of variable traits. When different natural genotypes of the same species are directly compared, heritable variation is observed for the number, size, shape, distribution or colour of particular cell types, such as trichomes and stomata

(Hausser *et al.,* 2001; Sun *et al.,* 2003), or tissues like vascular ones (Candela *et al.,* 1999). Large variation is also detected for the size, shape, number, organization and colour pattern of the basic plant organs (roots, leaves and stems) and of more complex structures (flowers, fruits and seeds). In addition, considerable differences are found for the number, distribution and activity of meristems, which determine the number and length of lateral branches and inflorescences, the architectural growth pattern and the different types of growth habit like the degree of erectness and determinacy. An endless list of intraspecific genetic differences have been recorded and classical examples in wild species are described by Griffiths and Gaders (1983), Linhart and Grant (1996) and Briggs and Walters (1997) or in domesticated and ornamental plant species by Darwin (1883 and 1884). Figures 1 and 2 illustrate some of the commonest developmental differences observed in *Arabidopsis thaliana* and *Phaseolus vulgaris*, a wild and a domesticated species respectively.

Naturally occurring size and shape differences in particular organs or structures such as roots (Beemster *et al*., 2002; Mouchel *et al*., 2004), leaves (Pérez-Pérez *et al.*, 2002; Tsukaya *et al*., 2002), seeds (Alonso-Blanco *et al*., 1999; Doganlar *et al*., 2000), or carpels and fruits (Tanksley, 2004) have been associated with variation in cell size and number. Detailed dynamic analysis of growth parameters like for instance root elongation in *Arabidopsis* (Beemster *et al*., 2002) shows that natural genetic variation affects cell expansion and cell production and that genetic differences in cell production affect cell cycle duration and the number of dividing cells. Cell production has been associated with cyclin-dependent kinase A (CDKA) amount and activity, suggesting that differences in expression, protein stability and other molecular regulatory mechanisms of CDKA activity contribute to root growth differences. Furthermore, differential effects of hormones such as auxins, brassinosteroids or gibberellins on the development of particular organs like adventitious roots or hypocotyls, have been also reported in different natural genotypes of *Arabidopsis* (King and Stimart, 1998; Maloof *et al*., 2001; Borewitz *et al*., 2002). Therefore, natural genetic differences of growth parameters affect a wide range of cellular mechanisms, including the morphogenic regulatory effects of plant hormones.

Variation for the temporal regulation of development

Plant development occurs continuously throughout the life cycle and variation among genotypes is also found for its temporal and ontogenetic control. This includes genetic differences for growth rate, initiation rate of particular organs like leaves (so-called plastochron), or for the duration or average age of organs and structures (Koornneef *et al*., 2004). Moreover, differences are commonly reported for the timing of certain developmental processes, such as those involved in the ontogenetic changes associated with heteroblasty, anther or fruit dehiscence, or sepal and petal abscission (Pérez-Pérez *et al*., 2002; Tsukaya, 2002). Specially interesting is the variation for the timing of major developmental transitions such as germination or flowering initiation (Figure 2A) because they establish the duration of the main developmental phases along the life cycle (growth arrest phase during seed dispersion, vegetative growth phase and reproductive growth phase). Such genetic variation determines the life habit of plants, particularly in monocarpic species, which can be classified in perennial, biennial or annual depending on the duration of their vegetative phase. Different varieties have been described in many wild and domesticated species with extreme life habits varying from perennial to biennial, from biennial to annual, or from winter annual (annual plants growing vegetatively during winter) to summer annual (annual plants that germinate at the end of winter or later) (Briggs and Walters, 1997; Koornneef *et al*., 2004). Polycarpous plants also show genetic variation for the duration of developmental phases, as described for instance for the length of the flowering period in the short-lived perennial *Fragaria vesca* (Albani *et al*., 2004), the timing of vegetative bud set in *Populus* (Frewen *et al*., 2004), or the timing of the first blooming in tree species (Hacket, 1985; Martin-Trillo and Martinez-Zapater, 2002). The latter defines the duration of the so-called juvenile vegetative phase of woody plants and it is associated with changes in vegetative development such as foliar form, density of thorns or branch features.

Variation for the environmental regulation of development

The expression of many developmental traits depends on the environmental conditions, which defines the unique plant developmental plasticity, *i.e.* the capacity of a genotype to express different phenotypes depending on the environment. Environmental factors affecting plant development include all kind of abiotic signals such as light, temperature, wind, humidity, water availability or nutrient resources as well as most biotic components of the surrounding environment from pathogens to competitors (Tonsor *et al*., 2005). Environmental context-dependency has been found for many of the developmental traits pointed above and for the temporal control of phenological features (Sultan, 2000; Casal *et al*., 2004). In addition, intraspecific genetic variation for the plasticity of developmental traits in relation to particular environmental factors has often been detected by testing genotype by environment (G x E) interactions (Sultan, 2000). Considerable variation has been found for instance, for shoot and root developmental changes related with nutrient and water availability (Malamy, 2005), or for the light regulated model plastic response so-called shade avoidance syndrome (reviewed in Schmitt *et al*., 2003). In the latter, plants respond to crowding and vegetation shade with heterophyllic changes such as petiole and leaf elongation, increased main shoot elongation, lateral shoot suppression and earlier flowering. It is suggested that genetic variation for these plastic responses reflect local adaptation to different heterogeneous environments (Schmitt *et al*., 2003).

Genetic control of natural variation

Some developmental polymorphisms found among natural genotypes of the same plant species correspond to qualitative dimorphisms, like for instance: the clock-/anticlock-wise coiling of pod fruits and the leaf colour pattern of *Medicago truncatula* (Thoquet *et al*., 2002); the actinomorphic/zygomorphic flowers differing between naturally occurring peloric and wild type forms of *Digitalis, Antirhrinum* or *Linaria* (Cubas *et al*., 1999a; Rudall and Bateman, 2003); the discoid or rayed capitulum inflorescence morphologies of composite *Senecio* species (Anderson 2001); the determinate/indeterminate inflorescence growth found in common bean and cucumber cultivars (Park *et al*., 1999; Fazio *et al*., 2003); the presence/absence of trichomes and the early/late

flowering found in some *Arabidopsis* accessions (reviewed in Koornneef *et al.*, 2004); the plastic response of flowering initiation in relation to photoperiod in some rice varieties (Yano *et al.*, 2001); or the seasonal/perpetual flowering of the short-life perennial *Fragaria vesca* (Albani *et al.*, 2004). Such discrete differences are often under monogenic or digenic control and have been studied by classical Mendelian genetic analysis (Figure 1 A, C and E). However, most naturally segregating developmental traits show a continuous quantitative phenotypic distribution within a species, *i.e.* they are quantitative traits (Figures 1 and 2). Continuous patterns of variation are determined by the combined effects of environment and several segregating genes and therefore are under multigenic control (reviewed in Barton and Keightley, 2002). Only in the past 15 years routine protocols have been developed to genetically identify and characterize the loci contributing to a quantitative trait, referred to as quantitative trait loci (QTL).

In addition to the qualitative and quantitative variation visible by direct comparison of genotypes, a large amount of hidden genetic variation is detected as trangressive segregation in crosses between different genotypes of wild or domesticated species (Rieseberg *et al.*, 2003a). Further hidden genetic variation can be found by comparing allele effects in different genetic backgrounds, which reflects the importance of genetic (epistatic) interactions in plant development. Such genetic-context dependency of gene effects is illustrated with the appearance in *Arabidopsis* of a "cauliflower like" phenotype when introgressing the *ap1* allele from one wild background into another (Kempin *et al.*, 1995). Similar differences have been also shown for the expression of the self-incompatibility (SI) trait in the self-fertile species *Arabidopsis thaliana* (Nasrallah *et al.*, 2004). In this case, the authors disclosed interesting variation at SI modifier genes when introducing by transgenesis the S-locus recognition genes from the self-incompatible *A. lyrata*.

In the following section we summarize current methodologies to identify the loci, the genes and the polymorphisms underlying qualitative and quantitative developmental variation.

Analysing the genetic and molecular basis of natural developmental diversity in model and non-model organisms

Forward analysis: genetic and genomic tools for QTL mapping and cloning

Quantitative genetic analysis of experimental segregating populations derived from intra- or inter-specific crosses allow the genetic dissection of developmental differences. For that, phenotypic and genotypic data obtained from the same population are statistically compared to identify QTLs causing variation (reviewed in Doerge, 2002). Such analyses aim to determine the genetic architecture of traits including: 1) the number of loci affecting a trait; 2) their approximate genome map position within a confidence genetic interval ranging from 5 to 50 cM (which corresponds to 1.2-12 Mb of DNA in small genomes like that of *Arabidopsis* or rice, up to the 9-90 Mb or 25-250 Mbs for the large genomes of maize and barley respectively); 3) estimates of relative and absolute additive effects and the mode of action of individual QTLs; 4) detection of significant genetic (epistatic) interactions among QTL and estimation of their effects; 5) detection of significant QTL by environment interactions. The latter information can be obtained when a trait is analysed in the same population grown in different environments and will identify QTLs accounting for variation for developmental plasticity. 6) Finally, detection of putative pleiotropic effects of QTLs on various traits, which can be hypothesized by comparative QTL mapping of several related or unrelated traits analysed on the same mapping population.

QTL mapping analyses are facilitated by current marker technologies that provide an almost unlimited number of molecular markers for the construction of genetic maps. In addition, genetic and genomic resources including permanent mapping populations of recombinant inbred or doubled haploid lines, marker/polymorphisms databases and high-throughput genotyping technologies exist for many plant species (Borevitz and Nordborg, 2003; Borevitz and Chory, 2004; Koornneef *et al.*, 2004). QTL mapping studies are not limited anymore to crop species such as maize (Stuber,1995), tomato (Tanksley, 2004), rice (Yano *et al.*, 2001), or wheat (Yan *et al.*, 2003; Yan *et al.*, 2004a) where the theoretical framework of QTL mapping was derived (Tanksley, 1993), or model wild plants like *Arabidopsis thaliana* (Alonso-Blanco and Koorneef, 2000; Koorneef *et al.*, 2004) and *Medicago truncatula* (Thoquet *et al.*, 2002). QTL mapping has become a routine analytical tool in plant genetics and progresses and caveats for the analysis of wild and domesticated species have been reviewed elsewhere (e.g. Kearsey and Farquhar, 1998; Mauricio, 2001; Erickson *et al.*, 2004). Developmental traits have been dissected in a wide range of species including for instance: leaf features in the European beech tree (*Fagus sylvatica* L.) (Scalfi *et al.*, 2004) or the pasture legume *Stylosanthes scabra* (Thumma *et al.*, 2001); branching pattern in cassava (*Manihot esculenta* Crantz) (Okogbenin and Fregene, 2003); wood properties in loblolly pine (*Pinus taeda* L.) (Brown *et al.*, 2003); or growth traits in Douglas fir (*Pseudotsuga menziesii* Mirb.) (Jermstad *et al.*, 2003) and chestnut (*Castanea sativa* Mill.) (Casasoli *et al.*, 2004). Broader genetic variation has also been analysed in interspecific crosses aiming to identify the genetic basis of plant domestication and plant diversification and speciation. Important contributions include the pioneering and extensive analyses of maize plant and ear architectures (Dorweiler *et al.*, 1993; Doebley, *et al.*, 1997; Lauter and Doebley 2002; Doebley, 2004) and of tomato fruit and seed morphologies (Doganlar *et al.*, 2000; Friedman *et al.*, 2004; Tanksley, 2004). Recent studies further show the extended power of QTL mapping as illustrated with the analysis of developmental traits related with domestication of wheat species (Faris *et al.*, 2003; Peng *et al.*, 2003); morphological traits related to ecological divergence of sunflowers (Rieseberg *et al.*, 2003b); fibre characteristics in *Gossypium* (Paterson *et al.*, 2003); leaf and flower features in *Rhododendron* (Dunemann *et al.*, 1999), *Rosa* (Crespel *et al.*, 2002) and *Linaria* hybrids (Tsiantis and Hay, 2003); bud setting and flushing in *Populus* (Frewen *et al.*, 2004); floral traits in relation to mating system in *Lycopersicon* (Chen and Tanksley, 2004), *Mimulus* (Bradshaw *et al.*, 1995; Lin and Ritland, 1997) and *Iris* species (Mauricio, 2001); or microsporangium number in *Microseris* (Gailing and Bachmann, 2003).

In contrast to the feasibility of QTL mapping studies, zooming into naturally segregating loci (of quantitative or qualitative effect) to find their molecular bases is not straightforward and lags far

behind. The identification of genes underlying QTLs is referred to as QTL cloning. This requires first, the fine mapping of QTLs to smaller regions spanning a reduced number of candidate genes. Thereafter, several functional genomic strategies can be applied to find the precise gene and the nucleotide polymorphisms affecting gene function, here referred to as functional polymorphisms (reviewed in Borevitz and Nordborg, 2003; Borevitz and Chory, 2004; Koornneef *et al.*, 2004). QTL candidate genes within a restricted DNA segment may be selected based on: 1) gene functions predicted in genome sequences; 2) phenotypic analysis of mutants available in public collections or newly generated; 3) transcriptomic analyses; 4) association studies of DNA polymorphisms in candidate genes (described in the following section); 5) association analyses of nucleotide polymorphisms linked to QTL by using linkage disequilibrium mapping; 6) genetic complementation in transgenic plants. These approaches are powerfully improved by the resources derived from genome projects in model plant species (reviewed in Borevitz and Ecker, 2004). Such resources include genomic and expressed sequence tag databases; sequence polymorphism databases (Bazin *et al.*, 2005); whole genome collections of full-length cDNA; large sets of knockout mutants available in T-DNA, transposson or TILLING services (Henikoff and Comai, 2003) and whole genome arrays. The latter is enabling RNA expression analyses at the genome level (Schadt *et al.*, 2003), which is a particularly promising approach to characterise developmental QTLs since transcriptional regulation is a major component of developmental variation. In this way, *cis*- and *trans*- regulatory elements affecting gene expression, so-called expression QTLs (eQTL) can be studied in relation to particular developmental differences (Kirst *et al.*, 2004).

The molecular bases of naturally segregating loci have been identified only for a very limited number of examples in model species (Morgante and Salamini, 2003; Paran and Zamir, 2003). Current plant genetic models are the domesticated species rice, maize, tomato, *Brassica rapa* or *Populus trichocarpa* and the wild plants *Arabidopsis thaliana* and *Medicago truncatula*. However, other wild species chosen because they belong to phylogenetically distant families and genera such as *Mimulus*, *Aquilegia* or *Selaginella* are becoming genetic systems (Borevitz and Ecker, 2004) and new ones will join in the near future (Feder and Mitchell-Olds, 2003). These species show different life habits, they are ecologically wide spread and they contain considerable developmental intraspecific and/or intragenus variation amenable to dissection by forward genetic analysis. It is expected that the exploitation of these model organisms will provide an unprecedented frame for the molecular analysis of natural developmental diversity.

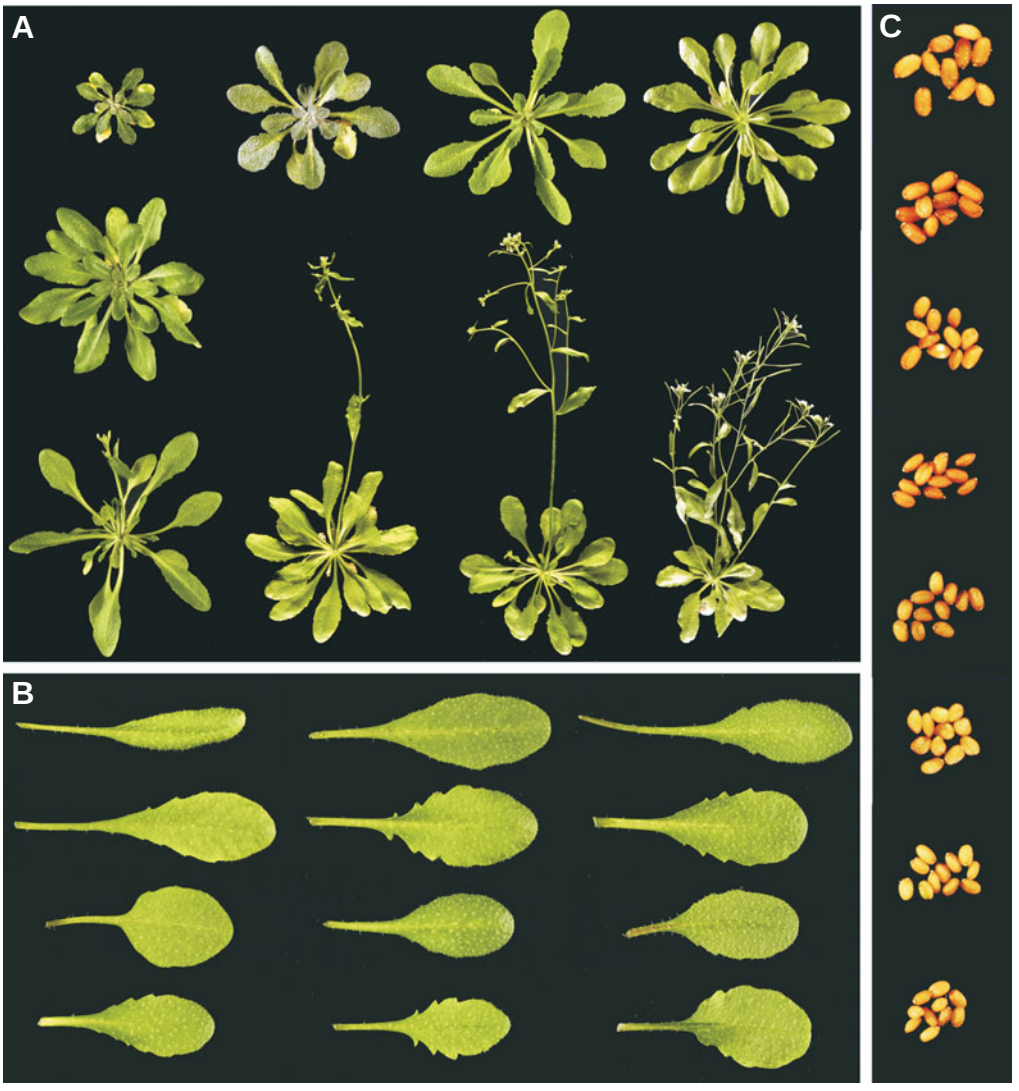

Fig. 2. Developmental variation in the wild species *Arabidopsis thaliana* L. *Wild genotypes of* Arabidopsis *differ quantitatively for the following traits: vegetative phase length (or flowering time), growth rate (plastochron), morphology of the rosette and morphology of the inflorescence* **(A)***; size, shape, serration and trichome density of leaves and petioles* **(B)***; size and colour of seeds* **(C)***. Plants were grown together and samples were collected from comparable nodes, at the same age; pictures in each panel are at the same magnification.*

Reverse analyses: association studies with candidate genes

To identify genes and polymorphisms determining natural variation, research can be focused on specific selected genes. To this end, it can be directly evaluated if natural molecular variation might be associated with phenotypic variation. Essentially, the effect of nucleotide polymorphisms detected in multiple alleles segregat-

ing among different natural or domesticated populations may be statistically tested in so-called association analyses. These studies mainly require gene sequencing and phenotypic analyses, which can be easily applied on model and non-model species. Such approach is particularly straightforward in plant species closely related to model systems because DNA sequence information and genomic resources might be easily transferred due to the relative low divergence. In addition, association analyses require taking into account the genetic structure of the population of genotypes that are compared by including neutral molecular markers. The potential usefulness of these studies has been shown with the maize *Dwarf8* gene where 9 polymorphisms spanning a 3.6 kbs region were associated with flowering time and suggested a causal relationship (Thornsberry *et al.*, 2001). Genes can be selected based on their function known from previous mutant or natural variation studies. This is illustrated with the association analyses performed in *Arabidopsis* with the *GL1* gene involved in trichome development (Hauser *et al.*, 2001) and the *CRY2*, *FRI* and *FLC* flowering time genes (Caicedo *et al.*, 2004; Hagenblad *et al.*, 2004; Olsen *et al.*, 2004). These studies can also be performed to test candidate genes underlying a QTL, as shown with the association of the *COL1* gene of *Brassica nigra* with flowering time (Österberg *et al.*, 2002). It is expected that large-scale association analyses with sets of selected genes affecting a particular trait will allow the detection of putative genes accounting for natural intraspecific variation. However, formal demonstration of functional and phenotypic effects of the genes and polymorphisms significantly associated will require further experimentation.

Molecular basis of natural allelic variation affecting plant development

In the past ten years, the strategies described above have been applied to dissect genetically several developmental traits up to the identification of the causal gene and the functional nucleotide polymorphism. The gene and nucleotide polymorphism underlying a QTL are often referred to as QTG (quantitative trait gene) and QTN (quantitative trait nucleotide) respectively. However, this nomenclature should not reflect a simplistic molecular view of naturally segregating loci. This is illustrated with the identification of clusters of small gene families corresponding to a single QTL affecting a physiological trait (Kroymann *et al.*, 2003) and to the qualitative effect *I* locus controlling seed colour in soybean (Tuteja *et al.*, 2004). In these cases, several tandem repeated genes contribute to the phenotypic effect and may interact genetically. In addition, changes in gene function underlying a naturally segregating locus might often not be assigned to a single nucleotide, but to several polymorphisms acting in an additive or interacting manner. This has been shown in *Drosophila* by association analysis, where an aminoacid polymorphism and two polymorphisms in non-coding regions of the *Dopa-decarboxilase* gene interact epistatically to affect fly longevity (De Luca *et al.*, 2003). Currently, about 30 genes have been involved in natural variation for particular developmental processes in different plant species (see Table 1). Most studied genes affect quantitative traits, although their allelic variation shows large discrete phenotypic effect. Extensive systematic studies have been performed on two developmental aspects, flowering time and fruit morphology and composition, which have become model quantitative traits. Flowering time has been thoroughly analysed in several species of two plant families. In contrast, fruit traits have been mainly studied in tomato related species and some other *Solanaceae*. Furthermore, the molecular analysis of other morphological traits has begun in several other species. Here we describe the genes and functional nucleotide polymorphisms presently identified, classified according to their main developmental effect.

Fruit and seed variation in Solanaceae *and other species*

Fruit morphology and composition have been dramatically modified during tomato domestication from its wild ancestor, to increase fruit yield. Numerous quantitative genetic analyses have been carried out to identify the loci that account for fruit morphological differences observed within and between *Lycopersicon* species (reviewed in Tanksley, 2004). Comparative studies involving multiple crosses indicate that around 30 loci are enough to explain most fruit morphological diversity. The genes underlying two of these loci, *FW2.2* and *OVATE*, have been isolated, both revealing novel proteins with limited homology to previously known proteins (Frary *et al.*, 2000; Liu *et al.*, 2002). *FW2.2* affects fruit size and encodes a transmembrane protein that negatively regulates cell division during the early stages of fruit development. The precise mutations that affect fruit size remain unknown, but detailed sequence and expression analyses indicate that these are not protein changes and that they modulate the level and timing of transcript expression. Thus, it has been suggested that one or several of eight nucleotide polymorphisms found in *FW2.2* promoter region are regulatory polymorphisms (Cong *et al.*, 2002). *OVATE* affects fruit shape and encodes a nuclear localised protein that is speculated to negatively regulate carpel and fruit growth (Liu *et al.*, 2002). All pear-shaped or elongated tomato varieties carry the same loss-of-function allele generated by a nucleotide substitution resulting in a premature stop codon. In addition, QTLs affecting fruit size and shape have been mapped around the genetic positions of orthologous genes in eggplant and pepper. Therefore, it has been suggested that these two genes may account for the variation in other *Solanaceae* species (Tanksley, 2004).

On the other hand, tomato yield related traits have been dissected using a genome-wide population of introgression lines derived from the highly divergent interspecific cross *Solanum lycopersicum* x *S. pennellii* (Fridman *et al.*, 2004). In this way, more than 25 loci have been identified affecting fruit sugar content. Among those, only *Brix9-2-5* has been molecularly studied and it encodes the flower and fruit specific invertase LIN5. Functional analysis of *Brix9-2-5* allelic variation has demonstrated that a single aminoacid substitution nearby the catalytic site affects enzyme activity and sugar content (Fridman *et al.*, 2004).

Further natural diversity of fruit developmental characteristics is currently being analysed in other important crop species that belong to different taxonomical families such as *Vitacea* and *Fabaceae*. This is illustrated with the analysis of fruit and seed traits in grapevine, soybean and pea. Recently, the *VvmybA1* transcriptional regulator has been found as a gene underlying variation for the colour of berry skins in grapevine (Kobayashi *et al.*, 2004). White-skinned varieties carry a homozygous

TABLE 1

A CATALOGUE OF NATURALLY SEGREGATING GENES AND FUNCTIONAL NUCLEOTIDE POLYMORPHISMS AFFECTING PLANT DEVELOPMENT

Taxonomic Family	Plant species		Developmental trait	Pleitropic effects	Variation analysed	Locus/Gene	Molecular function	Functional nucleotide polymorphism		Allelic dysfunction	References
								Intragenic position	Mutation		
Poaceae	Rice	*Oryza sativa*	Flowering time		intra	*Hd1*	CO like TF	coding	Del (2 bp) TE insertion	Truncated protein Misexpression	Yano *et al.*, 2000 Doi *et al.*, 2004
			Flowering time		intra	*Hd3a*	FT like protein	-	-	Misexpression	Kojima *et al.*, 2002
			Flowering time		intra	*Hd6*	Protein kinase CK2	coding	Nonssense Subs	Truncated protein	Takahashi *et al.*, 2001
			Flowering time		inter, intra	*Ehd1*	Response regulator	coding	Missense Subs	Altered protein	Doi *et al.*, 2004
	Einkorn wheat	*Triticum monococum*	Flowering time		intra	*VRN1*	AP1-like MADS TF	promoter [2]	Del[2]	Misexpresion	Yan *et al.*, 2003
			Flowering time		intra	*VRN2*	ZCCT1 (CO related TF)	promoter+coding, coding	Missense Subs Large Del	Altered protein Null expression	Yan *et al.*, 2004a
	Bread wheat	*Triticum aestivum*	Flowering time		intra	*VRN1*	AP1-like MADS TF	promoter [2]	Del[2], Ins[2]	Misexpresion	Yan *et al.*, 2004b Trevaskis *et al.*, 2003
			Plant architecture	Plant stature, leaf size	intra	*RhtB1, RhtD1*	GAI-like putative TF	coding	Nonssense Subs	Truncated proteins	Peng *et al.*, 1999
			Inflorescence morphology	Free-threshing Floret morphology	inter	*Q*[2]	AP2 like TF	coding[2]	Missense Subs[2]	Altered protein[2]	Faris *et al.*, 2003
	Barley	*Hordeum vulgare*	Flowering time		inter, intra	*VRN2*	ZCCT1 (CO related TF)	promoter+coding	Large Dels	Null expression	Yan *et al.*, 2004a
			Floret morphology		intra	*HOODED*	BKnox3 TF	4th intron	Ins	Overexpression	Muller *et al.*, 1995
	Maize	*Zea mays*	Plant stature	Flowering time	intra	*Dwarf8* [1]	GAI-like putative TF	coding	Dels	Truncated protein	Peng *et al.*, 1999
			Flowering time		intra	*Dwarf8* [2]	GAI-like putative TF	promoter[2], coding[2]	Subs[2], Ins[2], Del[2]	-	Thornsberry *et al.*, 2001
			Plant architecture	Apical dominance Lateral meristem dev. Sexulaity of inflorescence	inter	*TB1*	TCP domain TF	promoter	-	Misexpression	Doebley *et al.*, 1997 Wantg *et al.*, 1999 Clark *et al.*, 2004
			Plant architecture	Apical dominance	inter	*QTL 3L/BA1*[2]	bHLH domain TF	promoter[2], coding[2]	-	-	Gallavotti *et al.*, 2004
Vitaceae	Grapevine	*Vitis vinifera*	Fruit skin color		intra	*VvmybA1*	MYB TF	promoter	TE insertion	Misexpression	Kobayashi *et al.*, 2004
			Plant stature	Tendril development Trichome density	intra	*VvGAI* [1,2]	GAI-like putative TF	coding[2]	Missense Subs	Altered protein[2]	Boss *et al.*, 2002
Fabaceae	Soybean	*Glycine max*	Seed color		intra	*I/CHS cluster*	Chalchone synthase	promoter[2], non-coding[2]	Ins[2], Del[2]	Misexpression	Tuteja *et al.*, 2004
	Pea	*Pisum sativum*	Seed form	Seed sugar, lipid and protein composition	intra	*R/SBEI*	Starch branching enzyme	coding	TE insertion	Truncated protein Misexpression	Bhattacharyya *et al.*, 1990
Brassicaceae	Thale cress (Arabidopsis)	*Arabidopsis thaliana*	Flowering time		intra	*FRI*	Unknown	promoter, coding	Ins, Del Ins,Del,Indel (1-345 bp)	Truncated protein Misexpression	Johanson *et al.*, 2000 Le Corre *et al.*, 2002
			Flowering time		intra	*FLC*	MADS TF	1st intron	TE insertion	Misexpression	Michaels and Amasino, 1999 Michaels *et al.*, 2003
			Flowering time	Seedling growth Fruit size	intra	*EDI/CRY2*	CRY2 photoreceptor	coding coding[2]	Missense Subs Missense Subs[2]	Altered protein Altered protein[2]	El-Assal *et al.*, 2001 Olssen *et al.*, 2004
			Flowering time		intra	*FLW1/FLM*	MADS TF	promoter+coding	Del (6.8 Kb)	Null expression	Werner *et al.*, 2005
			Flowering time	Sedling growth	intra	*PHYD*	PHYD photoreceptor	coding	Del (14 bp)	Truncated protein	Aukerman *et al.*, 1997
			Flowering time		intra	*HUA2* [1]	RNA processing	coding	Nonssense Subs	Truncated protein	Doyle *et al.*, 2005
			Seedling growth	Hypocotyl length	intra	*PHYA*	PHYA photoreceptor	coding	Missense Subs	Altered protein	Maloof *et al.*, 2001
			Seedling growth	Hypocotyl length Flowering time	intra intra	*PHYD*	PHYD photoreceptor	coding	Del (14 bp)	Truncated protein	Aukerman *et al.*, 1997
			Seedling growth	Cotyledon unfolding Flowering, fruit size	intra	*EDI/CRY2*	CRY2 photoreceptor	coding	Missense Subs	Altered protein	Botto *et al.*, 2003
			Inflorescence morphology		intra	*CAL* [1]	MADS TF	coding[2]	Missense Subs[2]	Altered protein[2]	Kempin *et al.*, 1995
			Ovule number/Fruit size	Flowering time, Seedling growth	intra	*EDI/CRY2*	CRY2 photoreceptor	coding	Missense Subs	Altered protein	El-Assal *et al.*, 2003
			Root growth		intra	*BRX*	Novel TF	coding	Nonssense Subs	Truncated protein	Mouchel *et al.*, 2004
	Turnip	*Brassica rapa*	Flowering time		intra	*BrFLC1*[2] and *BrFLC2*[2]	MADS TF	-	-	-	Kole *et al.*, 2001 Schranz *et al.*, 2002
	Black mustard	*Brassica nigra*	Flowering time		intra	*BniCOa*[2] or *BniCOL1*[2]	CO like TF	-	-	-	Osterberg *et al.*, 2002
	Cauliflower	*Brassica oleracea*	Inflorescence morphology		intra	*BoCAL*[2]	MADS TF	coding	Nonssense Subs	Truncated protein	Kempin *et al.*, 1995 Purugganan *et al.*, 2000
Solanaceae	Tomato	*Solanum lycopersicum*	Fruit size		inter	*FW2.2*	Transmembrane protein	promoter	Subs[2], indels (1-10 bp)[2]	Misexpression	Frary *et al.*, 2000 Cong *et al.*, 2002
			Fruit shape		intra	*OVATE*	Nuclear protein	coding	Nonssense Subs	Truncated protein	Liu *et al.*, 2002
			Fruit sugar content		inter, intra	*Brix9-2-5/LIN5*	Invertase	coding	Missense Subs	Altered protein	Fridman *et al.* 2004
			Plant architecture	Apical dominance Leaf morphology	intra	*Me/TKn2, LeT6* [1]	Knox 1 type TF	promoter	Gene fussion	Overexpression	Chen *et al.*, 1997 Parnis *et al.*, 1997
Scrophullariaceae	Toadflax	*Linaria vulgaris*	Floral simetry		intra	*Lcyc* [1]	TCP TF	promoter+coding	Hypermethilation	Misexpression	Cubas *et al.*, 1999a

Some genes and polymorphisms that do not contribute substantially to the species phenotypic diversity (1) as well as those whose phenotypic effects have not been formally demonstrated (2) are also included. Source of variation indicates whether the phenotypic differences studied are intraspecific (intra) or interspecific (inter). Size of plant drawings is proportional to the amount of molecular information currently available in each species. Subs: Substitutional mutation; Del: Deletion; Ins: Insertion; TE: Transposable element; TF: Transcription factor; Indel: Complex simultaneous insertion and deletion; -: Unknown polymorphism.

[1] Genes showing natural allelic variation of phenotypic effect but not contributing to the phenotypic variation among cultivated or wild genotypes due to: recent spontaneous origin and/or obvious deleterious effects (*Dwarf8*, *HUA2*, *Me*, *Lcyc*); chimaerism (*VvGAI*); or genetic interaction (*CAL*); (see text for more details).

[2] Loci or nucleotide polymorphisms associated with phenotypic variation but lacking causal demonstration.

retrotranposon insertion at the promoter region of *VvMybA1*, which leads to gene silencing. In contrast, red-skinned cultivars derived by bud mutation from white skinned ones are heterozygous for a reversion allele showing a transposable element (TE) recombination footprint. It is suggested that this moderately frequent white-skinned allele has a unique origin and was extensively spread throughout the cultivation area. Thus, natural variation for grape skin colour appears generated by TE insertion as well as reverted excisions.

A more complex molecular mechanism is speculated for the regulation of colour of soybean seeds (Tuteja *et al.*, 2004). Unlike wild soybean accessions, cultivated varieties have yellow seed coats that lack accumulation of anthocyanins. This difference is genetically controlled by the *I* (*Inhibitor*) locus, which corresponds to a tandem inverted repeat of three chalchone synthase (CHS) genes. Dominant alleles at the *I* locus inhibit seed coat pigmentation by silencing the expression of CHS genes at other loci in a trans-dominant manner. Several spontaneous recessive *i* pigmentation alleles that release the trans-silencing mechanism have been found, which are associated with independent deletions within the *I*-CHS cluster. It is hypothesized that this trans-silencing effect is mediated by double stranded RNA (dsRNA) transcribed from the inverted repeat present at the *I* locus (Tuteja *et al.*, 2004). Derivative short interfering RNA (siRNA) would then trigger sequence specific RNA degradation and gene trans-silencing. Hence, domestication of soybean some 10.000 years ago involved the acquisition of such duplicated CHS silencing alleles, which illustrates the complex evolution of multigene families and clusters of tandem repeated genes.

At last, the classical pea *R* (*RUGOSUS*) locus described by Mendel as affecting seed form has been also molecular characterised (Bhattacharyya *et al.*, 1990). This locus shows pleiotropic effects on sugar, lipid and protein composition of seeds and encodes a starch-branching enzyme (SBEI). The recessive *r* allele was caused by insertion of a transposon–like element in the coding sequence, which has been widely dispersed among European commercial cultivars since Mendel time due to the sweetness of wrinkled peas (Bhattacharyya *et al.*, 1990). This study exemplifies how metabolism can play a central role in plant development, since the *R* locus affects primarily starch biosynthesis and subsequently, seed composition, osmotic pressure, cell size and the final dry seed shape.

Flowering time variation in Brassicaceae *and* Poaceae *species*

The timing of flowering is an important adaptive trait because it synchronises reproduction with the most favourable season of the year for it. In addition, it is a plastic developmental feature since it is strongly affected by many seasonally-fluctuating environmental factors differing throughout the earth, such as photoperiod (daylength) and winter temperature (Koornneef, *et al.*, 1998; Simpson and Dean, 2002; Hayama and Coupland, 2004). The vast intraspecific genetic variation found for flowering time in wild monocarpic species is presumed to reflect adaptations to different environments. Furthermore, this trait has been genetically manipulated by human selection in many crop species to derive varieties locally adapted to different agricultural environments.

The regulation of flowering time is a relatively well known plant developmental process, mainly due to the study of artificially induced mutants in *Arabidopsis*. It has been shown that the differentiation of the apical meristem from vegetative to reproductive phase is determined by several meristem identity genes. A complex network of flowering induction pathways that integrate the signals from the various environmental factors, regulate the expression and function of meristem identity genes (Koornneef, *et al.*, 1998; Simpson and Dean, 2002). More than 80 genes have been involved in these pathways, either as promoters or repressors of flowering (or as repressors or promoters of vegetative development, respectively). Recently, large efforts have been focused to identify the molecular bases of natural variation for flowering time in the two main model systems, *Arabidopsis* and rice, as well as in crops species of *Brassicaceae* and *Poaceae*. Interestingly, all these plants differ in their flowering responses to environmental factors, which allows a comparative analysis of the mechanisms determining within and between species diversification.

Arabidopsis is a facultative long-day (LD) species because it flowers faster when grown under LD than short-day (SD) photoperiods. In addition, most late flowering wild genotypes of *Arabidopsis* accelerate their flowering when exposed to a long period of cold that emulate winter growth, so-called vernalization. However, wild genotypes of *Arabidopsis* differ in their response to both environmental factors. Analyses of 8 crosses derived between different *Arabidopsis* wild genotypes have identified a minimum number of 14 QTLs that contribute to its naturally occurring flowering variation (reviewed in Koornneef *et al.*, 2004; Werner *et al.*, 2005). Six loci have been isolated using different strategies: *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) involved in the vernalization response; *EARLY DAY LENGTH INSENSITIVE* (*EDI=CRY2*), *PHYTOCHROME D* (*PhyD*) and *FLOWERING1* (*FLW1=FLM*) affecting the photoperiod response; the *ENHANCER OF ELF4 (EEL=HUA2)* locus affecting both responses (Table 1).

The isolation of *FRI* and *FLC* has revealed two novel genes that operate as flowering repressors, the laboratory early flowering strains carrying natural loss-of-function alleles of them. *FLC* encodes a transcription factor belonging to the MADS box family and its expression is down-regulated by vernalization (Michaels and Amasino, 1999). In contrast, FRI is a protein sharing no homology with previously known proteins that positively regulates *FLC* expression (Johanson *et al.*, 2000). Natural alleles of *FRI* and *FLC* interact synergistically to affect flowering and account for a considerable proportion of the flowering time and vernalization requirement variation found between summer and winter annual genotypes of *Arabidopsis*. At least eight putative null alleles of *FRI* have been described. One of them is caused by an indel in the promoter region, whereas 7 carry deletions in the coding sequence leading to in frame premature stop codons (Le Corre *et al.*, 2002; Gazzani *et al.*, 2003; Stinchcombe *et al.*, 2004). Some of these are rare alleles suggesting that they have been generated recently. However, the relatively high frequency of two deletion alleles (>10%) suggests that they are older. Unlike *FRI*, known dysfunction alleles of *FLC* are neither null nor frequent. Three loss-of-function alleles have been described, all of them resulting in lower *FLC* expression (Gazzani *et al.*, 2003; Michaels *et al.*, 2003). Two of them have been shown to carry independent natural insertions of transposable elements in the first intron. Interestingly, the TE insertion of one of these *FLC* alleles has been shown to cause partial silencing through DNA methylation of the TE and histone methylation in the surrounding *FLC* region (Liu *et al.*, 2004). These methylation

events are mediated by siRNAs generated from homologous TE copies in the genome and presumably create an island of heterochromatin that reduces gene expression (Liu *et al.*, 2004). Thus, differential mutational origin and geographic distribution of natural loss-of-function alleles at these two genes have contributed to the modification of vernalization responses in *Arabidopsis*.

In contrast to *FRI* and *FLC*, *EDI* and *FLW1* were first detected as QTLs and their isolation has identified novel alleles of genes previously known to regulate flowering time (El-Assal *et al.*, 2001; Werner *et al.*, 2005). In addition, the function of *PhyD* could be first established trough the analysis of natural variants (Aukerman *et al.*, 1997). *PhyD* behaves as flowering repressor while *EDI=CRY2* functions as promoter, both encoding apoproteins of a red and a blue light photoreceptor, respectively. *FLW1* encodes an *FLC* related transcription factor of the MADS family that also represses flowering. The novel natural alleles recently characterised in these three genes reduce flowering photoperiod sensitivity and exist at very low frequency, which suggests a recent origin or involvement in specific local adaptation. Loss-of-function alleles of *PhyD* and *FLW1* have deletions of different size and dysfunction. Whereas a 14 bps deletion produces absence of PhyD protein (Aukerman *et al.*, 2001), a 6.8 Kbs deletion removing the complete *FLW1* transcribed region eliminates gene expression (Werner *et al.*, 2005). In contrast, a strong gain of function allele has been found at *EDI=CRY2*, which is produced by a single aminoacid substitution (El-Assal *et al.*, 2001). An additional weak but frequent allele of *CRY2* differing in 8 aminoacid substitutions has been suggested by association studies (Olsen *et al.*, 2004).

One last gene, *HUA2*, has been found contributing to flowering variation among *Arabidopsis* wild genotypes (Doyle *et al.*, 2005). *HUA2* encodes a protein involved in RNA processing of several MADS box genes, such as *FLC* and *FLM*, which may explain its effects on both flowering responses to vernalization and photoperiod. A natural loss-of-function mutation has been found as an early flowering allele in one of the main laboratory wild-type strains (Doyle *et al.*, 2005). However, this allele has been only detected in some lines derived from the original wild genotype and therefore, it is unclear whether the mutation comes from a natural heterogeneous genotype or it was generated in the laboratory. Further analysis to identify this or other alleles in different wild genotypes is necessary to establish the contribution of *HUA2* to the naturally occurring flowering variation of *Arabidopsis*. Nevertheless, the fixation of this allele in some laboratory stocks is probably caused by artificial selection during laboratory plant growth, illustrating the likely unintentional "domestication" of laboratory strains.

Genetic variation has also commonly found within other crucifer species such as *Brassica sps*, especially for the vernalization requirement to induce flowering. Phenotypic extremes corresponding to annual and biennial varieties have been used to map two flowering time *Brassica rapa* QTLs whose location overlap with *FLC*-like genes (Schranz *et al.*, 2002). Further expression analysis suggested that *BrFLC* genes contribute to the vernalization response variation through a molecular mechanism analogous to that described in *Arabidopsis* (Kole *et al.*, 2001). Similar comparative QTL mapping studies in *B. nigra* have suggested that the homologues of the *Arabidopsis CO* gene, *BniCO* and *BniCOL1*, might be involved in flowering variation. Association of a *BniCOL1* polymorphic indel with flowering time differences further supported its contribution (Österberg *et al.*, 2002).

Naturally occurring variation for flowering time has been also dissected in two domesticated *Gramineae* species, rice and wheat, differing in their flowering responses to photoperiod and vernalization. Rice is a facultative SD plant since it flowers earlier in SD than LD photoperiod conditions. In addition, it does not show vernalization response, which is in agreement with the absence in the rice genome of orthologues of several *Arabidopsis* genes involved in flowering induction by low temperature (Izawa *et al.*, 2003). Several QTL mapping analyses have been performed in segregating populations derived from intraspecific crosses between varieties of the two domesticated subspecies, *Oryza sativa* ssp *japonica* and *O. sativa* ssp *indica* and from interspecific crosses between those and some wild relative species such as *O. glaberrima* (Yano *et al.,* 2001; Doi *et al.*, 2004). Thus, at least 14 QTLs have been mapped affecting flowering and photoperiod response in rice. Four of these loci have been isolated, three of them, *Hd1*, *Hd3a* and *Hd6*, identifying respectively orthologues of the *Arabidopsis CO, FT* and *CK2* genes known to participate in the regulation of flowering by photoperiod (Yano *et al.*,2000; Takahashi *et al.*, 2001; Kojima *et al.*, 2002). In contrast, *Ehd1* has revealed a B type response regulator without obvious orthologous gene in the *Arabidopsis* genome (Doi *et al.*, 2004). Natural null and loss-of-function alleles of these genes have been generated by deletion and TE insertion as well as by nonsense and missense mutations (Table 1). Analyses of these alleles show that *Hd3a* function as an activator of flowering that is up-regulated in SD conditions by *Hd1* and *Ehd1*, while under LDs its expression is down-regulated by *Hd1* and probably by *Hd6* too. Functional comparative studies of these genes with those of *Arabidopsis* are deciphering the molecular mechanisms that distinguish the regulation of flowering by photoperiod in SD and LD plants (reviewed in Hayama and Coupland, 2004). In addition, these comparisons suggest that different genes determine natural variation for the flowering response to photoperiod in these two species.

Contrary to rice, wheat and barley are LD species and show variation for the requirement of vernalization to induce flowering, which distinguishes winter and spring varieties. Two major effect interacting loci, *VRN1* and *VRN2*, determine this response and have been recently isolated (Table 1). *VRN2* encodes ZCCT1, a putative transcription factor containing a CCT domain present also in the CO family of proteins. This locus identified a novel gene since the ZCCT subfamily of proteins is specific of grasses (Yan *et al.*, 2004a). *VRN1* encodes another transcription factor of the MADS box family that is an orthologue of *Arabidopsis AP1* (Yan *et al.*, 2003). *VRN2* shows analogous function to *FLC* because it behaves as a floral repressor whose expression is down-regulated by vernalization. In addition, VRN2 suppresses the expression of *VRN1*, which behaves as an activator of flowering that is up-regulated by vernalization. Loss-of-function alleles of *VRN2* produced by a single aminoacid substitution or an entire gene deletion have been found in einkorn wheat varieties with spring life habit. In contrast, most spring varieties of different wheat species with functional *VRN2* alleles, carry independent deletions or insertions in a promoter region of *VRN1* that might be recognised by VRN2 or by VRN2 regulated proteins (Table 1). Such dominant spring *VRN1* alleles with promoter changes probably lack *VRN2* mediated regulation, leading to a gain of function by constitutive expression. Comparison of wheat and *Arabidopsis* shows that different genes cause natural variation for the vernalization re-

sponse in these species, suggesting that temperate grasses and *Arabidopsis* developed different molecular pathways to regulate flowering in response to low temperature (Yan *et al.*, 2004a).

At last, flowering variation has been also genetically dissected in maize, another grass that similar to rice, is an overall facultative SD species showing considerable flowering variation in relation to photoperiod. More than 22 crosses have been used in QTL mapping studies and a comparative meta-analysis of these populations has estimated an unforeseen high number of 62 different consensus QTLs (Chardon *et al.*, 2004). Nevertheless, none of these loci has been isolated yet, but only several molecular polymorphisms in coding and non-coding regions of the *Dwarf8* gene have been associated with flowering time variation (Thornsberry *et al.*, 2001). *Dwarf8* is orthologous of *Arabidopsis GAI* and spontaneously arisen gain or altered function *Dwarf8* alleles have strong effect on flowering and plant stature (Peng *et al.*, 1999). However, to know if the natural allelic variation present in cultivated varieties cause flowering differences await demonstration.

Comparisons of the results obtained in these two divergent plant families show that the flowering responses to photoperiod and vernalization within each species are modified by allelic variation at different genes. The analysis of variation for both environmental flowering responses in distinct species identifies orthologous proteins with partly different function as well as unrelated proteins with analogous function. These results suggest that the regulation of flowering by similar environmental factors has been partly acquired through different genes and molecular mechanisms in diverged species. This distinct genetic structure is expected to be a major factor constraining differently the most recent natural modifications of plastic responses within each family and species.

Variation for other morphological traits

Natural variation for other developmental traits has been also analysed molecularly in *Arabidopsis* and cultivated species. Three morphological subjects have been mainly addressed: seedling emergence, modifications of plant architecture and changes in inflorescence or flower morphology.

Seedling emergence and root growth in Arabidopsis

When seeds germinate in darkness, seedling development is etiolated, which is distinguished by the elongation of the embryonic shoot (hypocotyl) and the folding of the embryonic leaves (cotyledons). This skotomorphogenic morphology is presumed to facilitate soil penetration and it is restrained by the light induced deetiolation. Photomorphogenic seedling development is characterised by inhibition of hypocotyl elongation and promotion of cotyledon expansion. This is affected by red (R), far-red (FR) and blue (B) lights and considerable genetic variation has been found among *Arabidopsis* wild genotypes for these plastic responses to different lights (Maloof *et al.*, 2001). QTL mapping analyses in an experimental population derived between two accessions differing in light responses show that variation for hypocotyl and cotyledon development was affected by distinct loci (Botto *et al.*, 2003). Hence, the various components of photomorphogenesis have been naturally modified through mutations at different genes with specific developmental effects. Currently, three loci affecting deetiolation have been isolated by different approaches and all have identified novel rare alleles of three major classes of photoreceptors: the red light photoreceptors PhyD and PhyA and the blue light photoreceptor CRY2 (Table 1). A loss-of-function deletion of *PhyD* reduces the R light mediated hypocotyl length inhibition. In contrast, gain-of-function alleles caused by aminoacid substitutions at PhyA and CRY2 increased the stability of both proteins under particular light regimes and affect different light regulated responses (El-Assal *et al.*, 2001; Maloof *et al.*, 2001). The novel *PhyA* allele altered hypocotyl length response to FR light due to changes in the photochemical and kinase properties of PhyA protein. In contrast, the new *CRY2=EDI* allele increased the B light mediated hypocotyl length inhibition but also increased the cotyledon unfolding mediated by FR pulses, even in the absence of blue light (Botto *et al.*, 2003). As described above, *PhyD* and *CRY2* affected also flowering time, suggesting that these natural photoreceptor alleles might also affect other light-mediated plastic responses like shade avoidance. In addition, the *CRY2* allelic variation shows pleiotropic effects on other apparently unrelated developmental traits such as fruit size and the number of ovules and seeds per fruit (El Assal *et al.*, 2004).

On the other hand, variation for root morphology has been also studied in *Arabidopsis* (Malamy, 2005). A major effect QTL affecting root length named as *BREVIS RADIX* (*BRX*) has been recently isolated leading to the identification of a novel family of putative transcription factors (Mouchel *et al.*, 2004). *BRX* controls root growth by affecting cell proliferation and cell elongation apparently, in an environment independent manner. A unique loss of function allele generated by a nonsense mutation has been found so far, but it will be very interesting to encounter other natural dysfunctional alleles of *BRX*.

Modifications of plant architecture in domesticated plants

Plant architecture has been dramatically modified during domestication and the subsequent improvement of many crop species. The molecular basis of some of these modifications has been recently identified in maize, wheat and tomato. Domestication of maize from its wild progenitor teosinte 5000-10000 years ago was characterised by increased apical dominance and reduced number of lateral branches; sexual transformation of the inflorescences at the tips of primary branches from male to female; and emergence of the polystichous and naked grained inflorescences with synchronised maturation (Iltis, 1983; Doebley, 2004). These modifications led to the rapid development of a highly productive and extremely easily harvestable new species unique for human nutrition. Several of these morphological differences are controlled by a major effect QTL, which was shown by genetic complementation to correspond to the *TEOSINTE BRANCHED1* locus (*TB1*). *TB1* has been isolated and encodes a TCP domain transcription factor that regulates developmental arrest of lateral meristems and sexual identity of secondary inflorescences (Doebley *et al.*, 1997; Cubas *et al.*, 1999b). *TB1* is over-expressed in maize compared to teosinte indicating that a regulatory change occurred between both species, although the functional polymorphism(s) remain unknown (Wang *et al.*, 1999). Extensive sequencing in the *TB1* genomic region has defined a selective sweep area spanning the 60-90 kbs 5´ to the transcribed region, which shows reduced genetic diversity in maize compared to teosinte. Thus, selection has operated on regulatory sequence(s) that might be located more distant than the presumed promoter region (Clark *et al.*, 2004). In addition, another locus involved in the modification of

plant architecture during maize domestication, QTL *3L*, has been recently speculated to correspond to the *BA1* gene encoding a transcription factor that regulates the expression of *TB1* (Gallavotti *et al*., 2004). This is supported by *BA1* sequence analysis and by the genetic interaction between QTL *3L* and *TB1* to regulate lateral branch development.

Striking morphological changes in plant architecture have been also recently selected for the improvement of wheat achieved during the "green revolution" taking place in the 1960s and 1970s. New bread wheat varieties were developed by selecting dwarf plants with reduced internode and leaf elongation, which increased grain yield and resistance to wind and rain damage. These modifications were caused by spontaneous mutations at the *RhtB1* or *RhtD1* loci that qualitatively reduced the responses to the plant hormone gibberellin. Both genes behave as growth repressors and are orthologues of the *Arabidopsis GAI* and maize *Dwarf8* genes encoding putative transcriptional regulators involved in gibberellin sensitivity (Peng *et al*., 1999). Dwarfing selected alleles are dominant and carry nonsense substitutions that encode truncated proteins. Such strong developmental modifications are still being selected and illustrate the most recent events in wheat domestication. On the other hand, a comparable natural allele has been found in the phylogenetically distant species *Vitis vinifera*. The grapevine cultivar Pinot Meunier developed in the 1500s appeared as a chimeric genotype that is heterozygous for this dominant allele in the L1 layer but not in L2 cells (Boss and Thomas, 2002). Interestingly, *VvGAI* effects on flowering and plant stature are only visible in non-chimerical plants regenerated from the L1 layer but not directly in Pinot Meunier plants.

Several other spontaneous mutations with multiple qualitative pleiotropic effects on plant architecture have been characterised in cultivated species but they do not contribute substantially to the phenotypic diversity present among cultivated or natural genotypes and therefore, to our broad view of natural variation. We will only describe the *Mouse ears* (*Me*) mutant of tomato isolated in the 1950s because it illustrates another molecular mechanism to naturally modify plant development. *Me* is a dominant allele showing reduced apical dominance, altered inflorescence architecture and increased leaf compoundness (Chen *et al*., 1997; Parnis *et al*., 1997). All these effects are due to overexpression of *Tkn2*, a Knox transcriptional regulator (Table 1). It has been shown that the *Me* gain-of-function allele was generated by duplication of a house keeping gene encoding a phosphofructokinase (*PFP*) and its fusion to the 5´end of *Tkn2*. Thus, a *PFP-Tkn2* hybrid transcript with different expression pattern is generated by a new combination of different coding and regulatory genomic modules.

Changes in inflorescence and flower morphology of cultivated plants

Inflorescence and flower morphology have been often modified in cultivated species and the molecular bases of some changes have been established in cauliflower, wheat, barley and *Linaria vulgaris* (Table 1).

Morphological selection during *Brassica oleracea* domestication led to distinct subspecies such as *B. oleracea* ssp *botrytis* (cauliflower) and *B. oleraceae* ssp *italica* (broccoli). These are characterised by an indeterminate and undifferentiated growth of reproductive meristems. This phenotype has been associated with a single nonsense mutation found in the MADS box gene *CAL* of both subspecies (Kempin *et al*., 1995; Purugganan *et al*., 2000). However, though this mutation is almost fixed in these subspecies, it has been also found in other non-cauliflower like subspecies. Therefore, this mutation is not sufficient to produce the cauliflower phenotype. Another natural loss of function *CAL* allele has been found in the related species *Arabidopsis*, the function of *Arabidopsis CAL* in inflorescence development being partially redundant with *AP1* (Kempin *et al*., 1995). Hence, mutations in other MADS box related genes probably contribute to the cauliflower phenotype of *B. oleracea*.

A major morphological change during wheat domestication is the squared headedness and free-threshing phenotype of the spikes that facilitates grain harvest. This phenotype is mainly controlled by the *Q* locus, which pleiotropically affects several other floral traits of agronomical interest. The *Q* allele present in cultivated varieties of bread wheat is partly dominant, while null alleles behave similar to the *q* alleles of hulled non-free-threshing wild wheats. Detailed fine mapping and sequencing have identified an *AP2* like gene as a likely candidate for *Q* (Faris *et al*., 2003).

Another floral modification of recent natural origin is caused in barley by the *Hooded* mutation. A single dominant allele produces the development of an extra flower at the transition between the lemma and the awn (Muller *et al*., 1995). This distinct allele appeared only once at the end of the 18th century in the Himalaya region but spread world-wide since then. *Hooded* encodes the homeobox transcription factor BKnox3, its transcript being overexpressed from the *Hooded* mutant allele. Sequence association analysis showed that a duplication of 305 bps in the 4th intron is the cause of the gain of function, probably due to transcriptional regulatory elements present within that region (Santi *et al*., 2003). Interestingly, this barley epiphyllous flower formation from the lemma bract contrasts with the phenotype observed in *Me* tomato mutants, which shows again the differential effects of homologous genes in different species.

Finally, the classical naturally occurring peloric mutant of the wild species *Linaria vulgaris* shows transformation of the flower symmetry from bilateral to radial. This striking change has been shown to be due to a loss-of-function mutation at *Lcyc*, a homologue of the *Anthirrhinum CICLOIDEA* gene encoding a TCP transcription factor that controls dorsoventral asymmetry (Cubas *et al*., 1999a and 1999b). The peloric allele shows transcriptional silencing but no significant *Lcyc* DNA sequence polymorphism was found. Instead, peloria was associated with heavy methylation of *Lcyc* coding and regulatory regions. This transformation demonstrates the role of epigenetic mutations as another molecular mechanism to generate natural variation.

Molecular targets of plant developmental selection: gene position within networks and polymorphism location within genes

Construction of a descriptive catalogue of nucleotide functional polymorphisms that determine naturally occurring modifications of plant development is a first step to infer the molecular mechanisms involved in the evolution of development. The studies reported in Table 1 provide such a preliminary functional inventory within wild and domesticated species. However, several aspects limit the conclusions that may be currently drawn.

First, there is a reduced number of known polymorphisms and they are heterogeneous in terms of species, traits and relative effects of the mutations. Second, most current molecular studies have been performed in cultivated species where developmental modifications are derived from human selection under cultivation environments. Third, developmental differences have been molecularly studied only in one wild species, *Arabidopsis thaliana*. However, the effects of *Arabidopsis* variation on fitness and adaptation and the contribution of natural selection to this diversity may only be presumed.

Despite these limitations, a few conclusions can be inferred in relation to two important questions on naturally occurring intraspecific variation and the evolution of plant development: 1) what functional classes of genes are modified; 2) how those genes are changed, *i.e.*, which are the intragenic regions targeted presumably by selection, the coding or the regulatory modular segments (Doebley and Lukens, 1998). From a simple working perspective, molecular networks can be considered build up of hierarchical genetic pathways, which consist of sensors of signals (endogenous and environmental), primary and secondary signal transducers, transcriptional regulators and non-regulatory target effectors or structural genes. As shown in table 1, most of the polymorphisms presently reported affect transcriptional regulators. Thus, the analysis of intraspecific variation (and among closely related species) supports Doebley and Lukens (1998) proposition that transcriptional regulatory genes will be predominantly modified for the evolution of novel plant morphologies. Nevertheless, functional polymorphisms are also found in genes involved in environmental signal perception, like the *Arabidopsis* light photoreceptor genes *CRY2*, *PhyA* and *PhyD*, or in signal transduction, such as the rice genes *Hd3a* (related to mammalian membrane associated proteins) and *Hd6* (encoding a protein kinase). All these genes participate in the quantitative regulation of flowering, although not in the developmental process itself but in its environmental and temporal regulation. Therefore, modification of the environmental regulation of plastic traits may be achieved at different levels in developmental pathways. Such quantitative plasticity adds an extra layer in the molecular networks, probably distinguishing plant and animal development. In addition, polymorphisms have been also found in the putative signal transduction gene *FW2.2* involved in tomato fruit size and in enzyme encoding genes like *SBEI*, *CHS* or the invertase *Brix9-2-5*, affecting pea seed form, soybean seed colour, or tomato fruit sugar composition respectively (Table 1). Thus, different classes of genes are naturally targeted to change plant morphology and other developmental traits. Further understanding of target gene preferences within developmental networks requires a deep knowledge of the molecular organization of particular plant networks. These should integrate the precise position(s) of every molecular component and input signal, as well as their interactions (Cork and Purugganan, 2004; Tonsor *et al.*, 2005).

On the other hand, functional polymorphisms can affect either the coding region (altering protein structure) or the *cis*-regulatory non-coding segments of genes (leading to changes in transcript expression). Barely more than 50% of the intraspecific developmental polymorphisms identified so far involve protein coding regions, while the remaining polymorphisms imply regulatory changes altering gene expression. No obvious preference of intragenic location is observed for quantitative or qualitative effect polymorphisms, though most studied loci show large effect. However, polymorphisms on transcriptional regulator genes affect slightly more often their expression, whereas most polymorphisms on other genes affect protein structure. Again, current results from the analysis of intraspecific variation provide certain support to Doebley and Lukens (1998) postulation that there will be an overrepresentation of *cis*-regulatory changes in transcriptional regulators during the diversification of plant form among species. Nevertheless, protein structure polymorphisms are also frequently found in transcriptional regulators (*BRX, Ehd1, OVATE, RhtB1*) as well as some gene expression changes in other gene functional classes (*FW2.2*, *Hd3a*, *I* locus) (Table 1). Most regulatory mutations appear located in the 5´ regions of genes, probably affecting promoter or enhancer DNA motifs. However, polymorphisms in regulatory introns have been also found for some alleles of *FLC* and *Hooded*. On the other hand, most of the polymorphisms identified in coding regions are predicted to generate truncated proteins but polymorphisms altering protein sequence and properties are rather common (Table1). Furthermore, polymorphisms affecting both coding and regulatory regions are also contributing to within species developmental diversity as shown by the entire gene deletions of *VRN2* and *FLW1*.

From a mutational point of view, single nucleotide missense and nonsense substitutions are the commonest source to generate the naturally occurring variation analysed (*CRY2*, *PhyA*, *Ehd1*, *Hd6*, *BRX*, *OVATE* and *Brix9*). Small deletions also contribute to produce truncated proteins (*FRI* and *PhyD*). However, gene expression is mainly altered dramatically by small deletions in promoter regions (*FRI*, *VRN1*, *I* locus) or by larger deletions (*FLW1* and *VRN2*). More complex polymorphisms strongly affecting gene expression are produced by changes in promoter regions through either tandem duplication of a particular region (*I* locus), a duplication and fusion from another chromosomal region (*Me*), or by a TE insertion (*VvmybA1*). Alternatively, large gene expression modifications have been produced by mutations within regulatory introns such as duplications or TE insertions. All these deletion and insertion events might be generated by replication or recombination errors, or could be associated to transposition of TE elements. Interestingly, duplications of endogenous genes (*I* locus; Tuteja *et al.*, 2004) or transposons (*FLC*; Liu *et al.*, 2004) may both lead to siRNAs mediated silencing, which points to a common mechanism to generate genetic variation by duplications. Finally, naturally occurring silencing has been also produced by an epigenetic change like DNA methylation (*Lcyc*; Cubas *et al.*, 1999a).

As expected, the mutational mechanisms that naturally cause the genetic diversity that is maintained within species are also found in comparisons among species. However, these few examples cannot provide information on the role of factors constraining the molecular polymorphisms that are maintained intra- and interspecifically, such as: 1) features of the molecular network specifically regulating each developmental process and its plasticity; 2) relative phenotypic effects of the polymorphisms; 3) genome characteristics, including gene content, chromosomal organization and gene family distribution; 4) the different acting human or natural selective forces. To this end,

a much broader, systematic and detailed inventory of molecular functional polymorphisms is challenged.

Future prospects: genomic approaches in a functional ecological and evolutionary context

The analysis of natural variation in *Arabidopsis* and in cultivated species is providing an unprecedented knowledge of the molecular mechanisms involved in plant domestication and adaptation through developmental modifications. However, current studies are limited because they are mostly single gene and single trait analyses, performed in a limited number of species and mostly studied outside their natural environmental context. Expansion of several aspects in the research of intraspecific developmental diversity will help to overcome these limitations:

1) The analysis of variation in wild species is required to understand the evolution of plant development (Doebley and Lukens, 1998). This involves including other model wild species besides *Arabidopsis*, such as *Medicago*, *Mimulus*, *Aquilegia* or *Selaginella*, where transgenesis and other functional tools necessary to test candidate polymorphisms will soon be available.

2) Genomic approaches will efficiently increase the catalogue of natural functional nucleotide polymorphisms (Feder and Mitchell-Olds, 2003). Intraspecific genomic comparative analyses at the levels of DNA sequence, transcriptome, proteome, metabolome and methylome are expected to provide a large amount of potential functional polymorphisms (Borevitz and Ecker, 2004). Detection of relevant polymorphisms will be facilitated by including population genetics tests (Feder and Mitchell-Olds, 2003). Genomic approaches will also allow testing the combinatorial effects of natural polymorphisms to find substantial genetic interactions and emergent properties.

3) Detailed phenotypic analyses are needed at the cellular and organism level to identify small effect QTLs and their underlying polymorphisms and to estimate the extent of pleiotropy. Distinction of pleiotropy versus close linkage awaits further attention to evaluate if genes accounting for developmental variation at related traits are organised in the same genomic region. This is illustrated with the study of several floral polymorphisms associated with mating systems in tomato species, which are controlled by the *se2.1* locus (Chen and Tanksley, 2004). Fine mapping of *se2.1* indicates that five closely linked genes are differentially involved in style length, stamen length and dehiscence, suggesting that *se2.1* might corresponds to a coadapted gene complex.

4) Relevant developmental traits need to be addressed in an ecological context including the environmental factors that affect trait expression as well as those factors through which natural and/or artificial selection operated (Tonsor *et al.*, 2005). For instance, comparative studies of the natural intraspecific variation existing throughout the environmental distribution range of a species may provide clues on the adaptive significance of developmental modifications and on the evolutionary mechanisms maintaining this variation. This is illustrated with the analysis of natural *FRI* and *FLC* polymorphisms of *Arabidopsis* in relation to its latitudinal distribution (Caicedo *et al.*, 2004; Stinchcombe *et al.*, 2004).

Systematic analyses of the molecular mechanisms determining natural intraspecific developmental variation in multiple species will enable broader comparative analyses. The mechanisms involved in analogous developmental modifications within different species might be compared to study genome constrains for adaptation. In parallel, similar genomic comparative analyses of developmental diversification among wild (closely and distantly related) plant species in relation to important environmental factors will extend the study of evolution of development to a macro-evolutionary level (Cronk, 2001; Feder and Mitchell-Olds, 2003). Ultimately, comparison of the molecular mechanisms involved in intra- and interspecific developmental diversification will provide an integrated functional perspective of the continuous evolution of plant development.

Acknowledgements

We thank Juan José Ferreira for kindly providing seeds of common bean and Xavier Picó for critical comments on the manuscript. The authors apologise to those researchers whose work has not been included due to space limitation. The authors work was funded by the EU program NATURAL (contract QLG2-CT-2001-01097) and the Spanish Ministerio de Educación y Ciencia (grant BIO2004-00533). C.A.-B. was supported by a Ramón y Cajal contract.

References

ALBANI, M.C., BATTEY, N.H. and WILKINSON, M.J. (2004). The development of ISSR-derived SCAR markers around the *SEASONAL FLOWERING LOCUS* (*SFL*) in *Fragaria vesca*. *Theor. Appl. Genet.* 109: 571-579.

ALONSO-BLANCO, C. and KOORNNEEF, M. (2000). Naturally occurring variation in Arabidopsis: an underexploited resource for plant genetics. *Trends Plant Sci.* 5: 22-29.

ALONSO-BLANCO, C., BLANKESTIJN-DE VRIES, H., HANHART, C.J. and KOORNNEEF, M. (1999). Natural allelic variation at seed size loci in relation to other life history traits of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 96: 4710-4717.

ANDERSSON, S. (2001). The genetic basis of floral variation in *Senecio jacobaea* (Asteraceae). *J. Hered.* 95: 409-414.

AUKERMAN, M.J., HIRSCHFELD, M., WESTER, L., WEAVER, M., CLACK, T., AMASINO, R. and SHARROCK, R.A. (1997). A deletion in the PHYD gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. *Plant Cell* 9: 1317-1326.

BARTON, N.H. and KEIGHTLEY, P.D. (2002). Understanding quantitative genetic variation. *Nat. Rev. Genet.* 3: 11-21.

BAZIN, E., DURET, L., PENEL, S. and GALTIER, N. (2005). Polymorphix: a sequence polymorphism database. *Nucleic Acids Res.* 33: D481-D484.

BEEMSTER, G.T.S., VUSSER, K.D., TAVERNIER, E.D., BOCK, K.D. and INZÉ. D. (2002). Variation in growth rate between Arabidopsis ecotypes is correlated with cell division and A-type cyclin-dependent kinase activity. *Plant Physiol.* 129: 854-864.

BHATTACHARYYA, M.K., SMITH, A.M., ELLIS, T.H.N., HEDLEY, C. and MARTIN, C. (1990) The wrinkled-seed character of pea described by Mendel is caused by a trasnposon-like insertion in a gene encoding starch-branching enzyme. *Cell* 60: 115-122.

BOREVITZ, J.O., MALOOF, J.N., LUTES, J., DABI, T., REDFERN, J.L., TRAINER, G.T., WERNER, J.D., ASAMI, T., BERRY, C.C., WEIGEL, D. and CHORY, J. (2002). Quantitative trait loci controlling light and hormone response in two accessions of *Arabidopsis thaliana*. *Genetics* 160: 683-696.

BOREVITZ, J.O. and NORDBORG, M. (2003). The impact of genomics on the study of natural variation in Arabidopsis. *Plant Physiol.* 132: 718-725.

BOREVITZ, J.O. and CHORY, J. (2004). Genomics tools for QTL analysis and gene discovery. *Curr. Opin. Plant Biol.* 7: 132-136.

BOREVITZ, J.O. and ECKER, J.R. (2004). Plant genomics: The third wave. *Annu. Rev. Genomics Hum. Genet.* 5: 443-477.

BOSS, P.K. and THOMAS, M.R. (2002). Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature* 416: 847-850.

BOTTO, J.F., ALONSO-BLANCO, C., GARZARON, I., SANCHEZ, R.A. and CASAL,

J.J. (2003). The Cape Verde Islands allele of cryptochrome 2 enhances cotyledon unfolding in the absence of blue light in Arabidopsis. *Plant Physiol.* 133: 1547-1556.

BRADSHAW, H.D. WILLBERT, S.M., OTTO, K.G. and SCHEMSKE, D.W. (1995). Genetic mapping of floral traits associated with reproductive isolation in monkey flowers (*Mimulus*). *Nature* 376: 762-765.

BRIGGS, D. and WALTERS, S.M. (1997) Plant variation and evolution. Cambridge University Press, Cambridge.

BROWN, G.R., BASSONI, D.L., GILL, G.P., FONTANA, J.R., WHEELER, N.C., MEGRAW, R.A., DAVIS, M.F., SEWELL, M.M., TUSKAN, G.A. and NEALE, D.B. (2003). Identification of quantitative trait loci influencing wood property traits in loblolly pine (*Pinus taeda* L.) III. QTL verification and candidate gene mapping. *Genetics* 164: 1537-1546.

CAICEDO, A.L., STINCHCOMBE, J.R., OLSEN, K.M., SCHMITT, J. and PURUGGANAN, M.D. (2004). Epistatic interaction between Arabidopsis FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. *Proc. Natl. Acad. Sci. USA* 101: 15670-15675.

CANDELA, H., MARTINEZ-LABORDA, A. and MICOL, J.L. (1999). Venation pattern formation in *Arabidopsis thaliana* vegetative leaves. *Dev. Biol.* 205: 205-216.

CASAL, J.J., FANKHAUSER, C., COUPLAND, G. and BLAZQUEZ, M.A. (2004). Signalling for developmental plasticity. *Trends in Plant Sci.* 9: 309-314.

CASASOLI, M., POT, D., PLOMION, C., MONTEVERDI, M.C., BARRENECHE, T., LAUTERI, M. and VILLANI, F. (2004). Identification of QTLs affecting adaptative traits in *Castanea sativa* Mill. *Plant, Cell & Environment* 27: 1088-1101.

CHARDON, F., IRLON, B., OREAU, L., ALQUE, M., OETS, J., ECOUSSET, L., URIGNEUX, A. and HARCOSSET, A. (2004). Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. *Genetics* 168: 2169-2185.

CHEN, J.J., JANSSEN, B.J., WILLIAMS, A. and SINHA, N. (1997). A gene fusion at a homeobox locus: alterations in leaf shape and implications for morphological evolution. *Plant Cell* 9: 1289-1304.

CHEN, K.Y. and TANKSLEY, S.D. (2004). High-resolution mapping and functional analysis of se2.1: a major stigma exertion quantitative trait locus associated with the evolution from allogamy to autogamy in the genus Lycopersicon. *Genetics* 168: 1563-1573.

CLARK, R.M., LINTON, E., MESSING, J. and DOEBLEY, J.F. (2004). Pattern of diversity in the genomic region near the maize domestication gene tb1. *Proc. Natl. Acad. Sci. USA* 101: 700-707.

CONG, B., LIU, J. and TANKSLEY, S.D. (2002). Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations.n*Proc. Natl. Acad. Sci. USA* 99: 13606-13611.

CORK, J.M. and PURUGGANAN, M.D. (2004). The evolution of molecular genetic pathways and networks. *Bioassays* 26: 479-484.

CRESPEL, L., CHIROLLET, M., DUREL, E., ZHANG, D., MEYNET, J. and GUDIN, S. (2002). Mapping of qualitative and quantitative phenotypic traits in *Rosa* using AFLP markers. *Theor. Appl. Genet.* 105: 1207-1214.

CRONK, Q.C. (2001). Plant evolution and development in a post-genomic context. *Nat. Rev. Genet.* 2: 607-619.

CUBAS, P., VINCENT, C. and COEN, E. (1999a). An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401: 157-161.

CUBAS, P., LAUTER, N., DOEBLEY, J. and COEN, E. (1999b). The TCP domain: a motif found in proteins regulating plant growth and development. *Plant J.* 18: 215-222.

DARWIN, C. (1884). The different forms of flowers on plants of the same species. New York University Press, New York, 1989.

DARWIN, C. (1883). The variation of animals and plants under domestication. The Johns Hopkins University Press, London, 1998.

DE LUCA, M., ROSHINA, N.V., GEIGER-THORNSBERRY, G.L., LYMAN, R.F., PASYUKOVA, E.G. and MACKAY, T.F. (2003). Dopa decarboxylase (Ddc) affects variation in Drosophila longevity. *Nature Genetics* 34: 429-433.

DIAMOND, J. (2002). Evolution, consequences and future of plant and animal domestication. *Nature* 418: 700-707.

DOEBLEY, J. (2004). The genetics of maize evolution. *Annu. Rev. Genet.* 38: 37-59.

DOEBLEY, J., STEC, A. and HUBBARD, L. (1997). The evolution of apical dominance in maize. *Nature* 386: 485-488.

DOEBLEY, J. and LUKENS, L. (1998). Transcriptional regulators and the evolution of plant form. *Plant Cell* 10: 1075-1082.

DOERGE, R.W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. *Nat. Rev. Genet.* 3: 43-52.

DOGANLAR, S., FRARY, A. and TANKSLEY, S.D. (2000). The genetic basis of seed-weight variation: tomato as a model system. *Theor. Appl. Genet.* 100: 1267-1273.

DOI, K., IZAWA, T., FUSE, T., YAMANOUCHI, U., BO, T., IMATANI, Z., YANO, M. and YOSHIMURA A. (2004). *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes Dev.* 18: 926-936.

DORWEILER, J., STEC, A., KERMICLE, J. and DOEBLEY, J. (1993). Teosinte glume architecture 1: A genetic locus controlling a key step in maize evolution. *Science* 262: 233-235.

DOYLE, M.R., BIZZELL, C.M., KELLER, M.R., MICHAELS, S.D., SONG, J., NOH, Y.S. and AMASINO, R.M. (2005). *HUA2* is required for the expression of floral repressors in *Arabidopsis thaliana*. *Plant J.* 41: 376-385.

DUNEMANN, F., KAHNAU, R. and STANGE, I. (1999). Analysis of complex leaf and flower characters in *Rhododendron* using a molecular linkage map. *Theor. Appl. Genet.* 98: 1146-1155.

EL-ASSAL, S.E.-D., ALONSO-BLANCO, C., HANHART, C.J. and KOORNNEEF, M. (2004). Pleiotropic effects of the Arabidopsis cryptochrome 2 allelic variation underlie fruit trait-related QTL. *Plant Biol.* 6: 370-374.

EL-ASSAL, S.E.-D., ALONSO-BLANCO, C., PEETERS, A.J., RAZ, V. and KOORNNEEF, M. (2001). A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. *Nature Genetics* 29: 435-439.

ERIKSON, D.L., FENSTER, C.B., STENØIEN, H.K. and PRICES, D. (2004). Quantitative trait locus analysis and the study of evolutionary process. *Mol. Ecol.* 13: 2505-2522.

FARIS, J.D., FELLERS, J.P., BROOKS, S.A. and GILL, B.S. (2003). A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. *Genetics* 164: 311-321.

FAZIO, G., STAUB, J.E. and STEVENS, M.R. (2003). Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. *Theor Appl Genet.* 107: 864-874.

FEDER, M.E. and MICHELL-OLDS, T. (2003). Evolutionary and ecological functional genomics. *Nat. Rev. Genet.* 4: 649-655.

FREWEN, B.E., CHEN, T.H., HOWE, G.T., DAVIS, J., ROHDE, A., BOERJAN, W. and BRADSHAW, H.D.Jr. (2004). Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. *Genetics* 154: 837-845.

FRARY, A., NESBITT, T.C., FRARY, A., GRANDILLO, S., VAN DER KNAAP, E., CONG, B., LIU, J., MELLER, J., ELBER, R., ALPERT, K.B. *et al.* (2000) *fw2.2* : A quantitative trait locus key to the evolution of tomato fruit size. *Science* 289: 85-88.

FRIDMAN, E., CARRARI, F., LIU, Y.S., FERNIE, A.R. and ZAMIR, D. (2004). Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* 305: 1786-1789.

GAILING, O. and BACHMANN, K. (2003). QTL mapping reveals a two-step model for the evolutionary reduction of inner microsporangia within the Asteracean genus *Microseris*. *Theor. Appl. Genet.* 107: 893-901.

GALLAVOTTI, A., ZHAO, Q., KYOZUKA, J., MEELEY, R.B., RITTER, M., DOEBLEY, J.F., PE, M.E. and SCHMIDT, R.J. (2004). The role of barren stalk1 in the architecture of maize. *Nature* 432: 630-635.

GAZZANI, S., GENDALL, A.R., LISTER, C. and DEAN, C. (2003). Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. *Plant Physiol.* 132: 1107-1114.

GRIFFITHS, A.J.F. and GANDERS, F.R. (1983) Wildflower genetics. Flight Press, Vancouver.

HACKETT, W.P. (1985). Juvenility, maturation and rejuvenation in woody plants. *Horticultural reviews* 7: 109-155

HAGENBLAD, J., TANG, C., MOLITOR, J., WERNER, J., ZHAO, K., ZHENG, H., MARJORAM, P., WEIGEL, D. and NORDBOR,G. M. (2004). Haplotype structure and phenotypic associations in the chromosomal regions surrounding two

Arabidopsis thaliana flowering time loci. *Genetics* 168: 1627-1638.

HAUSER, M.-T., HARR, B. and SCHLOTTERER,C. (2001). Trichome distribution in *Arabidopsis thaliana* and its close relative *Arabidopsis lyrata* : Molecular analysis of the candidate gene *GLABROUS1*. *Mol. Biol. Evol.* 18: 1754-1763.

HAYAMA, R. and COUPLAND, G. (2004). The molecular basis of diversity in the photoperiodic flowering responses of Arabidopsis and rice. *Plant Physiol.* 135: 667-684.

HENIKOFF, S. and COMAI, L. (2003). Single-nucleotide mutations for plant functional genomics. *Annu Rev Plant Biol.* 54: 375-01.

ILTIS, H.H. (1983). From Teosinte to Maize: The catastrophic sexual transmutation. *Science* 222: 886-894

IRISH, V.F. and BENFEY, P.N. (2004). Beyond Arabidopsis. Translational biology meets evolutionary developmental biology. *Plant Physiol.* 135: 611-614.

IZAWA, T., TAKAHASHI, Y., YANO, M. (2003). Comparative biology comes into bloom: genomics and genetic comparison of flowering pathways in rice and Arabidopsis. *Curr. Opinion Plant Biol.* 6: 113-20

JERMSTAD, K.D., BASSONI, D.L., JECH, K.S., RITCHIE, G.A., WHEELER, N.C. and NEALE, D.B. (2003). Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas fir. III. Quantitative trait loci-by-environment interactions. *Genetics* 165: 1489-1506.

JOHANSON, U., WEST, J., LISTER, C., MICHAELS, S., AMASINO, R. and DEAN, C. (2000). Molecular analysis of *FRIGIDA*, a major determinant of natural variation in Arabidopsis flowering time. *Science* 290: 344-347.

KEARSEY, M.J. and FARQUHAR, A.G. (1998). QTL analysis in plants; where are we now? *Heredity* 80: 137-142.

KELLOGG, E.A. (2004). Evolution of developmental traits. *Curr. Opin. Plant Biol.* 7: 92-98.

KEMPIN, S.A., SAVIDGE, B. and YANOFSKY, M.F. (1995). Molecular basis of the cauliflower phenotype in Arabidopsis. *Science* 267: 522-525.

KING, J.J. and STIMART, D.P. (1998). Genetic analysis of variation for auxin-induced adventitious root formation among eighteen ecotypes of *Arabidopsis thaliana* L. Heynh. *Genetics* 89: 481-487.

KIRST, M., MYBURG, A.A., DE LEON, J.P., KIRST, M.E., SCOTT, J. and SEDEROFF, R. (2004). Coordinated genetic regulation of growth and lignin revealed by quantitative trait locus analysis of cDNA microarray data in an interspecific backcross of eucalyptus. *Plant Physiol.* 135: 2368-2378.

KOBAYASHI, S., GOTO-YAMAMOTO, N. and IROCHIKA, H. (2004). Retrotransposon-induced mutations in grape skin color. *Science* 304: 982.

KOJIMA, S., TAKAHASHI, Y., KOBAYASHI, Y., MONNA, L., SASAKI, T., ARAKI, T. and YANO, M. (2002). *Hd3a*, a rice ortholog of the Arabidopsis *FT* gene, promotes transition to flowering downstream of Hd1 under short-day conditions. *Plant Cell Physiol.* 43: 1096-1105.

KOLE, C., QUIJADA, P., MICHAELS, S.D., AMASINO, R. and OSBORN, T.C. (2001). Evidence for homology of flowering-time genes *VFR2* from *Brassica rapa* and *FLC* from *Arabidopsis thaliana*. *Theor. Appl. Genet.* 102: 425-430.

KOORNNEEF, M., ALONSO-BLANCO, C., PEETERS, A.J.M. and SOPPE, W. (1998). Genetic control of flowering time in Arabidopsis. *Ann. Rev. Plant Physiol.* 49: 345-370.

KOORNNEEF, M., ALONSO-BLANCO, C. and VREUGDENHIL, D. (2004). Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu. Rev. Plant. Biol.* 55: 141-172

KROYMANN, J., DONNERHACKE, S., SCHNABELAUCH, D. and MITCHELL-OLDS, T. (2003). Evolutionary dynamics of an Arabidopsis insect resistance QTL. *Proc. Natl. Acad. Sci. USA*. 100, 14587-14592

LAUTER, N. and DOEBLEY, J. (2002). Genetic variation for phenotypically invariant traits detected in teosinte: implications for the evolution of novel forms. *Genetics* 160: 333-342.

LE CORRE, V., ROUX, F. and REBOUD, X. (2002). DNA polymorphism at the *FRIGIDA* gene in *Arabidopsis thaliana* : extensive nonsynonymous variation is consistent with local selection for flowering time. *Mol. Biol. Evol.* 19: 1261-1271.

LIN, J.Z. and RITLAND, K. (1997). Quantitative trait loci differentiating the outbreeding *Mimulus guttatus* from the inbreeding *M. platycalyx*. *Genetics* 146: 1115-1121.

LINHART, Y.B. and GRANT, M.C. (1996). Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. of Ecol.Syst.* 27: 237-277.

LIU, J., VAN ECK, J., CONG, B. and TANKSLEY, S.D. (2002). A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. *Proc. Natl. Acad. Sci. USA* 99: 13302-13306.

LIU, J., HE, J., AMASINO, R. and CHEN, X. (2004). siRNAs targeting an intronic transposon in the regulation of natural flowering behavior in Arabidopsis. *Genes Dev.* 18: 2873-2878.

MALAMY, J.E. (2005). Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell and Env.* 28: 67-77.

MALOOF, J.N., BOREVITZ, J.O., DABI, T., LUTES, J., NEHRING, R.B., REDFERN, J.L., TRAINER, G.T., WORLAND, A.J., ASAMI, T., BERRY, C.C., *et al.* (2001). Natural variation in light sensitivity of Arabidopsis. *Nature Genetics* 29: 441-446.

MARTÍN-TRILLO, M. and MARTÍNEZ-ZAPATER, J.M. (2002) Growing up fast: Manipulating the generation time of trees. *Curr. Opin. Biotech.* 13: 151-155.

MAURICIO, R. (2001). Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nat. Rev. Genet.* 2: 370-381.

MICHAELS, S.D. and AMASINO, R.M. (1999). Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* 13: 935-941.

MICHAELS, S.D., HE, Y., SCORTECCI, K.C. and AMASINO, R.M. (2003). Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 100: 10102-10107.

MORGANTE, M. and SALAMINI, F. (2003). From plant genomics to breeding in practice. *Cur. Opin. Biotech.* 14: 214-219.

MOUCHEL, C.F., BRIGGS, G.C. and HARDTKE, C.S. (2004). Natural genetic variation in Arabidopsis identifies *BREVIS RADIX*, a novel regulator of cell proliferation and elongation in the root. *Genes Dev.* 18: 700-714.

MÜLLER, K.J., ROMANO, N., GERSTNER, O., GARCÍA-MAROTO, F., POZZI, C., SALAMINI, F. and ROHDE, W. (1995). The barley *Hooded* mutation caused by a duplication in a homeobox gene intron. *Nature* 374: 727-730.

NASRALLAH, M.E., LIU P., SHERMAN-BROYLES, S., BOGGS, N.A. and NASRALLAH, J.B. (2004). Natural variation in expression of self-incompatibility in *Arabidopsis thaliana* : implications for the evolution of selfing. *Proc. Natl. Acad. Sci. USA* 1019: 16070-16074.

OKOGBENIN, E. and FREGENE, M. (2003). Genetic mapping of QTLs affecting productivity and plant architecture in a full-sib cross from non-inbred parents in Cassava (*Manihot esculenta* Crantz). *Theor. Appl. Genet.* 107: 1452-1462.

OLSEN, K.M., HALLDORSDOTTIR, S.S., STINCHCOMBE, J.R., WEINIG, C., SCHMITT J. and PURUGGANAN M.D. (2004). Linkage disequilibrium mapping of Arabidopsis *CRY2* flowering time alleles. *Genetics* 167: 1361-1369.

ÖSTERBERG, M.K., SHAVORS, YA, O., LASCOUX, M. and LAGERCRANTZ, U. (2002). Naturally occurring indel variation in the *Brassica nigra COL1* gene Is associated with variation in flowering time. *Genetics* 161: 299-306.

PARAN, I. and ZAMIR, D. (2003). Quantitative traits in plants: beyond the QTL. *Trends Genet.* 19: 303-306.

PARK, S., COYNE, D.P., BOKOSI, J.M. and STEADMAN, J.R. (1999). Molecular markers linked to genes for specific rust resistance and indeterminate growth habit in common bean. *Euphytica* 105: 133-141.

PARNIS, A., COHEN, O., GUTFINGER, T., HAREVEN, D., ZAMIR, D. and LIFSCHITZ E. (1997). The dominant developmental mutants of tomato, *Mouse-ear* and *Curl*, are associated with distinct modes of abnormal transcriptional regulation of a *Knotted* gene. *Plant Cell* 9: 2143-2158.

PATERSON, A.H., SARANGA, Y., MENZ, M., JIANG, C.X. and WRIGHT, R.J. (2003). QTL analysis of genotype x environment interactions affecting cotton fiber quality. *Theor. Appl. Genet.* 106: 384-396.

PENG, J., RICHARDS, D.E., HARTLEY, N.M., MURPHY, G.P., DEVOS, K.M., FLINTHAM, J.E., BEALES, J., FISH, L.J., WORLAND, A.J., PELICA, F. *et al.* (1999). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400: 256-261.

PENG, J., RONIN, Y., FAHIMA, T., RODER, M.S., LI, Y., NEVO, E. and KOROL, A. (2003). Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. *Proc. Natl. Acad. Sci. USA* 100: 2489-2494.

PURUGGANAN, M.D., BOYLES, A.L. and SUDDITH, J.I. (2000). Variation and

selection at the *CAULIFLOWER* floral homeotic gene accompanying the evolution of domesticated *Brassica oleracea*. *Genetics* 155: 855-862.

PÉREZ-PÉREZ, J.M., SERRANO-CARTAGENA, J. and MICOL, J.L. (2002). Genetic analysis of natural variations in the architecture of *Arabidopsis thaliana* vegetative leaves. *Genetics* 162: 893-915.

RIESEBERG, L.H., WIDMER, A., ARNTZ, A.M. and BURKE, J.M. (2003a). The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 358: 1141-1147.

RIESEBERG, L.H., RAYMOND, O., ROSENTHAL, D.M., LAI, Z., LIVINGSTONE, K., NAKAZATO, T., DURPHY, J.L., SCHWARZBACH, A.E., DONOVAN, L.A. and LEXER, C. (2003b). Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301: 1211-1216.

RUDALL, P.J. and BATEMAN, R.M. (2003). Evolutionary change in flowers and inflorescences: evidence from naturally occurring terata. *Trends Plant Sci.* 8: 76-82.

SANTI, L., WANG Y., STILE, M.R., BERENDZEN, K., WANKE, D., ROIG, C., POZZI C., MULLER K., MULLER J., ROHDE W. *et al*. (2003). The GA octodinucleotide repeat binding factor BBR participates in the transcriptional regulation of the homeobox gene *Bkn3*. *Plant J.* 34: 813-826.

SCALFI, M., TROGGIO, M., PIOVANI, P., LEONARDI, S., MAGNASCHI, G., VENDRAMIN, G.G. and MENOZZI, P. (2004). A RAPD, AFLP and SSR linkage map and QTL analysis in European beech (*Fagus sylvatica* L.). *Theor. Appl. Genet.* 108: 433-441.

SCHADT, E.E., MONKS, S.A., DRAKE, T.A., LUSIS, A.J., CHE, N., COLINAYO, V., RUFF, T.G., MILLIGAN, S.B., LAMB, J.R., CAVET, G. *et al.* (2003). Genetics of gene expression surveyed in maize, mouse and man. *Nature* 422: 297-302.

SCHMITT, J., STINCHCOMBE, J.R., HESCHEL, M.S. and HUBER, H. (2003). The adaptive evolution of plasticity: Phytochrome-mediated shade avoidance responses. *Integr. Comp. Biol.* 43: 459-469.

SHEPARD, K.A. and PURUGGANAN, M.D. (2002). The genetics of plant morphological evolution. *Curr. Opin. Plant Biol.* 5: 49-55.

SIMPSON, G.G. and DEAN, C. (2002) Arabidopsis, the rosetta stone of flowering time? *Science* 296: 285-289.

SCHRANZ, M.E., QUIJADA, P., SUNG, S.-B., LUKENS, L., AMASINO,R. and OSBORN, T.C. (2002). Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. *Genetics* 162: 1457-1468.

STINCHCOMBE, J.R., WEINIG, C., UNGERER, M., OLSEN, K.M., MAYS, C., HALLDORSDOTTIR, S.S., PURUGGANAN, M.D. and SCHMITT, J. (2004). A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene *FRIGIDA*. *Proc. Natl. Acad. Sci. USA* 101:4712-4717.

STUBER, C.W. (1995). Mapping and manipulating quantitative traits in maize. *Trends Genet.* 11: 477-481.

SULTAN, S.E. (2000). Phenotypic plasticity for plant development, function and life history. *Trends Plant. Sci.* 5: 537-542

SUN, B., DILCHER, D.L., BEERLING, D.J., ZHANG, C., YAN, D., KOWALSKI, E. (2003) Variation in *Ginkgo biloba* L. leaf characters across a climatic gradient in China. *Proc. Natl. Acad. Sci. USA* 100: 7141-7146.

TAKAHASHI, Y., SHOMURA, A., SASAKI, T. and YANO, M. (2001). *Hd6*, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. *Proc. Natl. Acad. Sci. USA* 98: 7922-7927.

TANKSLEY, S.D. (1993) Mapping polygenes. *Annu. Rev. Genet.* 27: 205-233.

TANKSLEY, S.D. (2004). The genetic, developmental and molecular bases of fruit size and shape variation in tomato. *Plant Cell* 16: S181-S189.

THOQUET, P., GHERARDI, M., JOURNET, E.P., KERESZT, A., ANE, J.M., PROSPERI, J.M. and HUGUET, T. (2002). The molecular genetic linkage map of the model legume *Medicago truncatula* : an essential tool for comparative legume genomics and the isolation of agronomically important gen. *BMC Plant Biol.* 2: I.

THORNSBERRY, J.M., GOODMAN, M.M., DOEBLEY, J., KRESOVICH, S., NIELSEN, D. and BUCKLER, E.S.4th. (2001). *Dwarf8* polymorphisms associate with variation in flowering time. *Nature* 28: 286-289.

THUMMA, B.R., NAIDU, B.P., CHANDRA, A., CAMERON, D.F., BAHNISCH, L.M. and LIU, C. (2001). Identification of causal relationships among traits related to drought resistance in *Stylosanthes scabra* using QTL analysis. *J. Exp. Bot.* 52: 203-214.

TONSOR, S.J., ALONSO-BLANCO, C. and KOORNNEEFF, M. (2005). Gene function beyond the single trait: natural variation, gene effects and evolutionary ecology in *Arabidopsis thaliana*. *Plant, Cell and Env.* 28: 2-20.

TREVASKIS, B., BAGNALL, D.J., ELLIS, M.H., PEACOCK, W.J. and DENNIS, E.S. (2003). MADS box genes control vernalization-induced flowering in cereals. *Proc. Natl. Acad. Sci. USA* 100: 13099-13104.

TSIANTIS, M. and HAY, A. (2003). Comparative plant development: the time of the leaf? *Nat. Rev. Genet.* 4: 169-180.

TSUKAYA, H. (2002). The leaf index: heteroblasty, natural variation and the genetic control of polar processes of leaf expansion. *Plant Cell Physiol.* 43: 372-378.

TUTEJA, J.H., CLOUGH, S.J., CHAN, W.C. and VODKIN, L.O. (2004). Tissue-specific gene silencing mediated by a naturally occurring chalcone synthase gene cluster in *Glycine max*. *Plant Cell* 16: 819-835.

WANG, R.L., STEC, A., HEY, J., LUKENS,.L. and DOEBLEY, J. (1999). The limits of selection during maize domestication. *Nature* 398: 236-239.

WERNER, J.D., BOREVITZ, J.O., WARTHMANN, N., TRAINER, G.T., ECKER, J.R., CHORY, J. and WEIGEL, D. (2005). QTL mapping and DNA array hybridization identify a deletion in the *FLC* homolog *FLM* as a cause for natural variation in flowering time of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 102:2460-2465.

YAN, L., LOUKOIANOV, A., TRANQUILLI, G., HELGUERA, M., FAHIMA, T. and DUBCOVSKY, J. (2003). Positional cloning of the wheat vernalization gene *VRN1*. *Proc Natl Acad Sci USA* 100: 6263-6268.

YAN, L., LOUKOIANOV, A., BLECHL, A., TRANQUILLI, G., RAMAKRISHNA, W., SANMIGUEL, P., BENNETZEN, J.L., ECHENIQUE, V. and DUBCOVSKY, J. (2004a). The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* 303: 1640-1644.

YAN, L., HELGUERA, M., KATO, K., FUKUYAMA, S., SHERMAN, J. and DUBCOVSKY, J. (2004b). Allelic variation at the *VRN1* promoter region in polyploid wheat. *Theor. Appl. Genet.* 109: 1677-1686.

YANO, M., KATAYOSE, Y., ASHIKARI, M., YAMANOUCHI, U., MONNA, L., FUSE, T., BABA, T., YAMAMOTO, K., UMEHARA, Y., NAGAMURA, Y. and SASAKI, T. (2000). *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. *Plant Cell* 12: 2473-2483.

YANO, M., KOJIMA, S., TAKAHASHI, Y., LIN, H. and SASAKI, T. (2001). Genetic control of flowering time in rice, a short-day plant. *Plant Physiol.* 127: 1425-1429.