Comparative biology comes into bloom: genomic and genetic comparison of flowering pathways in rice and *Arabidopsis*

Takeshi Izawa*, Yuji Takahashi† and Masahiro Yano‡

Huge advances in plant biology are possible now that we have the complete genome sequences of several flowering plants. Now, genomes can be comprehensively compared and map-based cloning can be performed more easily. Association study is emerging as a powerful method for the functional identification of genes and molecular genetics has begun to reveal the basis of plant diversity. Taking the flowering pathways as an example, we discuss the potential of several approaches to comparative biology.

Addresses
*‡National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba 305-8602, Japan
*e-mail: tizawa@nias.affrc.go.jp
†Institute of the Society for Techno-Innovation of Agriculture, Forestry, and Fisheries, Tsukuba 305-0854, Japan
‡e-mail: myano@nias.affrc.go.jp
Correspondence: Masahiro Yano

Current Opinion in Plant Biology 2003, **6**:113–120

This review comes from a themed issue on
Genome studies and molecular genetics
Edited by Takuji Sasaki and Ronald R Sederoff

1369-5266/03/$ – see front matter

DOI 10.1016/S1369-5266(03)00014-1

Abbreviations

APRR	*Arabidopsis PSEUDO RESPONSE REGULATOR*
CCA1	*CIRCADIAN CLOCK ASSOCIATED 1*
CK2	casein kinase2
CO	*CONSTANS*
COL1	*CONSTANS-LIKE1*
CRY	CRYPTOCHROME
ELF3	*EARLY FLOWERING3*
EMF2	*EMBRYONIC FLOWERING2*
FIE	*FERTILIZATION INDEPENDENT ENDOSPERM*
FKF1	*FLAVIN-BINDING, KELCH-REPEATS, F-BOX1*
FLC	*FLOWERING LOCUS C*
FT	*FLOWERING LOCUS T*
Hd1	*Heading date1*
LD	linkage disequilibrium
LFY	*LEAFY*
LHP1	*LIKE HETEROCHROMATIN PROTEIN1*
LHY	*LATE ELONGATED HYPOCOTYL*
GI	*GIGANTEA*
PHY	PHYTOCHROME
QTL	quantitative trait locus
SOC1	*SUPPRESSOR OF OVEREXPRESSION OF CO1*
TOC1	*TIMING OF CAB EXPRESSION1*
VRN1	*VERNALIZATION1*

Introduction

Recently, two complete plant genome sequences, one from a dicot and one from a monocot, have been reported [1••,2••,3]. This progress has opened a floodgate of comparative genomics and molecular genetics in flowering plants. We review the use of the extensive new genomic information to reveal genes that are related to floral induction. We discuss the recent progress of molecular genetics in uncovering a flowering pathway that is conserved between rice, a short-day plant, and *Arabidopsis*, a long-day plant. We also review a new approach to identifying natural variations in flowering-time genes, which have contributed to the speciation and diversity of flowering plants.

What do genome sequences tell us about flowering pathways?

The molecular genetics of the long-day plant *Arabidopsis* have revealed several pathways that regulate floral induction and related flowering-time genes [4,5]. A long-day promotion pathway, a gibberellic-acid promotion pathway, and vernalization and autonomous pathways are well characterized. Several flowering pathway integrators, into whose expression multiple environmental stimuli are 'integrated', have also been identified. In addition, genes that are involved in determining chromatin structure are also involved in floral induction. We reciprocally compared whole-genome sequences using the public database of the *indica* rice genome draft [1••] and the Münich Information Center for Protein Sequences (MIPS) *Arabidopsis* proteome database (http://mips.gsf.de/proj/thal/) to find genes that are orthologous to previously reported flowering-time genes (Table 1). (As this review is focused on comparative analyses, see the original papers in the reference list for more information on the biological functions of flowering-time genes.)

Photoreceptors that are involved in floral induction include phytochromes (PHY) and cryptochromes (CRY). *Arabidopsis* contains five *PHY* and two *CRY* genes, whereas rice contains three *PHY* and three *CRY* genes (possibly two *CRY1*-type and one *CRY2*-type) [6,7]. *PHYD* and *PHYE* are missing in rice. As well as these *PHY* and *CRY* genes, circadian clock genes are conserved between the two plant species. Each genome includes a *TIMING OF CHLOROPHYLL A/B BINDING PROTEIN* (*CAB*) *EXPRESSION 1* (*TOC1*) [8] and a *GIGANTEA* (*GI*) (or *OsGI*) [9,10,11•] orthologue. Orthologues of other members of the *Arabidopsis PSEUDO RESPONSE REGULATOR* (*APRR*) family, to which *TOC1* belongs, also exist in rice [12]. There are two *MYB*-related genes in the *Arabidopsis* circadian clock system, *LATE ELONGATED HYPOCOTYL* (*LHY*) [13] and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) [14], but only one orthologous gene,

Table 1

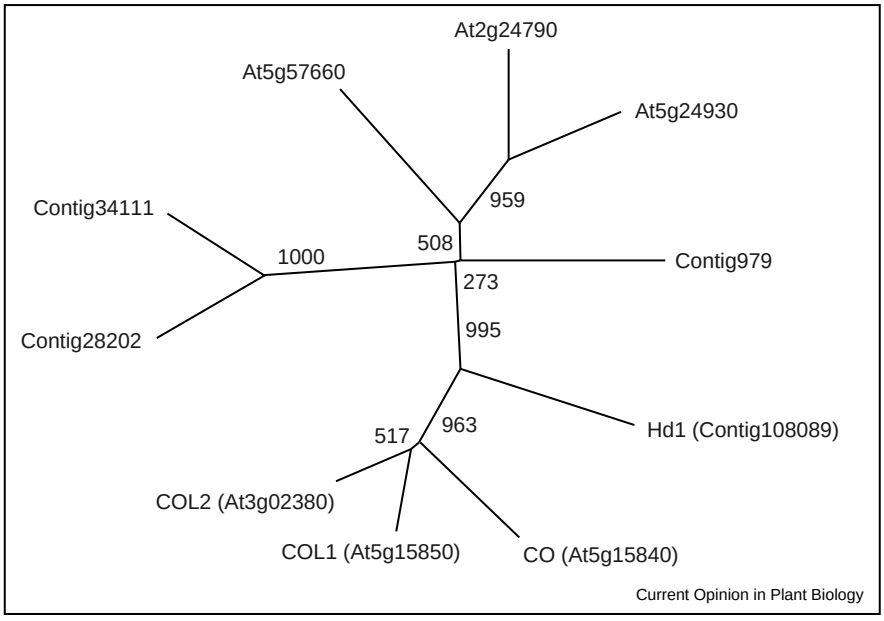
Rice genes that are orthologous to flowering-time genes of *Arabidopsis*.

Category	*Arabidopsis*	MIPS code	Rice	Contig No.	High scores vs. *Arabidopsis*†	vs. Rice‡	Possibility (N) vs. *Arabidopsis*†	vs. Rice‡
Photoreceptor	*PHY A*	At1g09570	*PHYA*	Contig2860	5754	2538	0(1)	0(4)
	PHY B	At2g18790	*PHYB*	Contig37882	6023	1757	0(1)	1.2e-295(2)
	PHY C	At5g35840	*PHYC*	Contig36094	5690	2299	0(1)	2.2e-267(2)
	CRY1	At4g08920		Contig7283	3610	1101	0(1)	3.1e-241(4)
				Contig27733		1351		8.7e-220(3)
				Contig21576*		405		2.5e-77(3)
				Contig10308*		481		3.0e-68(2)
	CRY2	At1g04400		Contig7283	3260	874	0(1)	1.8e-159(4)
				Contig27733		1066		7.4e-158(3)
				Contig21576*		448		1.9e-99(3)
				Contig10308*		522		4.2e-74(2)
Circadian clock	CCA1	At2g46830	*OsLHY*	Contig8936	3176	351	0(1)	1.9e-37(2)
	LHY	At1g01060	*OsLHY*	Contig8936	3353	424	0(1)	1.1e-45(2)
	GI	At1g22770	*OsGI*	Contig2215, 9556*	5982	1506, 314	0(1)	0(7), 3.8e-49
	TOC1/APRR1	At5g61380		Contig3599	3258	271	0(1)	3.0e-67(6)
	APRR3	At5g60100		Contig22039	2562	220	1.7e-267(1)	1.7e-37(4)
	APRR5	At5g24470		Contig6255	2882	185	2.1e-301(1)	1.4e-44(6)
	APRR7	At5g02810		Contig2278	3759	272	0(1)	1.6e-67(6)
	APRR9	At2g46790		Contig27958	2306	209	2.3e-240(1)	7.3e-38(5)
	ELF3	At2g25930		Contig9530	3669	220	0(1)	1.3e-42(4)
				Contig22380		228		1.1e-32(4)
	ZTL	At5g57360		Contig4859	3257	2371	0(1)	3.6e-261(2)
				Contig22611		2241		3.2e-245(2)
	LKP2	At2g18910		Contig4859	3202	1992	0(1)	1.8e-218(2)
				Contig22611		1929		1.1e-211(2)
Circadian-clock mediator	*FKF1*	At1g68050		Contig17935	3309	2222	0(1)	2.6e-230(1)
	CO	At5g15840	*Hd1*	Contig108089	1993	461	3.4e-207(1)	5.0e-43(1)
Floral pathway integrator	*FT*	At1g65480	*FTL/FT-L1*	Contig1060	933	338	7.1e-95(1)	3.9e-56(3)
			Hd3a/FT-L2	Contig2285*		326		1.2e-49(3)
			RFT1/FT-L3	Contig2285, 111231*		326, 151		1.2e-49(3), 7.5e-26(3)
			OsFT/FT-L4	Contig898, 75*		218, 180		3.3e-16(1), 3.6e-12(1)
			FT-L5	Contig3038		327		2.5e-54(3)
			FT-L6	Contig4876		322		5.7e-56(3)
			FT-L7	Contig10051		304		6.1e-49(3)
			FT-L8	Contig31550*		165		4.2e-19(2)
			FT-L9	Contig12831		223		5.1e-40(3)
			FT-L10	Contig4553		238		3.2e-42(10)
	SOC1	At2g45660		Contig2175	1077	267	3.9e-110(1)	1.6e-33(4)
	LFY	At5g61850	*RFL*	Contig1902	2270	511	1.5e-236(1)	1.6e-96(3)
Autonomous pathway	FCA	At4g18280		Contig1993	3959	230	0(1)	1.0e-64(11)
Chromatin-related	*EMF2*	At5g51230		Contig172	121	158	2.1e-160(1)	8.9e-68(14)
				Contig611, 1818*		146, 138		1.2e-32(10), 9.6e-24(4)
	FIE	At3g20740		Contig478	1973	187	4.4e-205(1)	3.8e-65(8)
				Contig88495		341		4.2e-30(1)
	LHP	At5g17690		Contig27271	2315	161	2.5e-241	2.2e-23(6)

*Genes are separated into several contigs. †*Arabidopsis* proteins were subjected to BLAST analysis with the MIPS *Arabidopsis* proteome database, indicating the values for the complete homology. ‡*Arabidopsis* proteins were subjected to BLAST analysis with the rice genomic contigs released by the Beijing group [1••]. Abbreviation: *LKP2*, *LOV KELCH PROTEIN2*.

OsLHY, in rice [15••]. At least one gene that is related to *Arabidopsis EARLY FLOWERING3* (*ELF3*) [16] has been found in rice, but no apparent orthologue of *ELF4* [17•] has been found in rice. Orthologues of both of the *Arabidopsis* F-box genes that function in the circadian clock, *ZEITLUPE* (*ZTL*) [18] and *FLAVIN-BINDING, KELCH-REPEATS, F-BOX1* (*FKF1*) [19], have been found in rice, suggesting that each of these genes has a unique biological role in addition to its function in the circadian clock. These results imply that a conserved

Figure 1



Phylogenetic tree for six *Arabidopsis* and four rice zinc-finger domains that are closely related to the CO protein (bootstrap values at nodes). CLUSTAL W software was used to make this tree.

flowering pathway is involved in photoreceptor and circadian-clock signaling rice and *Arabidopsis*, suggesting that this flowering pathway may exist in a large part of the plant kingdom.

The *Arabidopsis* genes *CONSTANS* (*CO*; a circadian-clock-related floral regulator [20]), *CONSTANS-LIKE1* (*COL1*), and *COL2* [21] belong to the same phylogenetic branch of a major quantitative trait locus (QTL) for flowering-time that is known as rice *Heading date1* (*Hd1*) ([22]; Figure 1). *COL1* and *COL2* do not appear to be involved in floral induction, but may be involved in circadian clock systems. Therefore, these genes may have acquired specific biological functions other than floral induction. The *Arabidopsis FLOWERING LOCUS T* (*FT*) gene [23,24] is both one of the target genes of *CO* and one of the integrators. *TWIN SISTER OF FT* in *Arabidopsis* and ten *FT-like* genes [15••] in rice, including *Hd3a* [25••] (another flowering-time QTL), belong to the same branch in a phylogenetic tree. The redundancy of *FT* orthologues in rice suggests that they have novel functions, but this remains to be examined. *Arabidopsis SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) [26], another direct target gene of *CO* and another integrator, is a member of the large MADS-box family. At least one rice orthologue of *SOC1* has been found on the basis of homology at the carboxy-end region as well as in the MADS and K domains. In addition, the *RICE FLO/LEAFY* (*RFL*) gene [27], which is present in the rice genome as a single copy, is orthologous to *LEAFY* (*LFY*), a single-copy gene that encodes another flowering pathway integrator in *Arabidopsis*.

FLOWERING LOCUS C (*FLC*) [28,29], a MADS-box gene, is a key regulator of the autonomous flowering and vernalization pathways in *Arabidopsis*. No apparent *FLC* orthologue has been found in rice. Neither has an orthologue of *Arabidopsis FRIGIDA* [30], the activator of *FLC*, been found in rice. Further, no orthologues of the recently identified *VERNALIZATION1* (*VRN1*) and *VRN2* genes [31••,32], which are required for vernalization in *Arabidopsis*, have been found in rice. It seems likely, therefore, that vernalization-related genes have been lost from the rice genome during evolution. This hypothesis is consistent with the fact that vernalization has not been reported in rice. FCA, an RNA-binding protein, is a component of the autonomous pathway that controls *FLC* expression [33]. A single orthologue of *FCA* has been found in the rice genome.

VRN2 belongs to the Polycomb group, whose members affect chromatin structure to repress gene expression. *EMBRYONIC FLOWERING2* (*EMF2*) [34] and *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*) [35•] also belong to the Polycomb group and affect floral induction in *Arabidopsis*. The *LIKE HETEROCHROMATIN PROTEIN1* (*LHP1*) [36•] gene may be involved in heterochromatin formation and affects flowering time in *Arabidopsis*. Two orthologues of *EMF2*, two orthologues of *FIE*, and an orthologue of *LHP1* have been found in

rice. These members of the Polycomb and heterochromatin protein groups affect the expression of multiple genes in a range of genomic regions. It will be interesting, therefore, to know whether the biological function of such chromatin-related genes is conserved among plant species, as gene order and chromosome structure are apparently not conserved between rice and *Arabidopsis*.

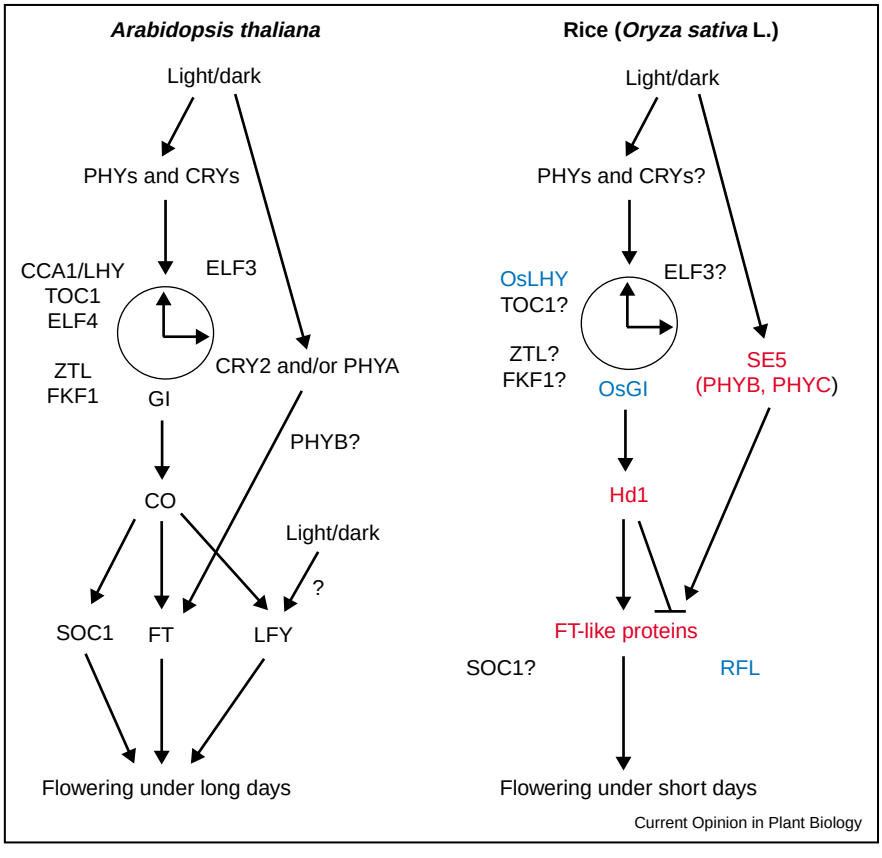
These comparisons of orthologous genes in rice and *Arabidopsis* provide many insights into the evolutionary conservation of flowering pathways in flowering plants. The functionality of many of these orthologues waits to be tested.

Flowering pathways conserved between short- and long-day plants

Recent progress in molecular genetics has provided evidence that supports the insights revealed by genomic comparison, revealing an evolutionarily conserved genetic pathway that regulates photoperiodic flowering in rice and *Arabidopsis*. Rice orthologues of the *Arabidopsis* genes *CO* and *FT* were recently identified as flowering-time QTLs named *Hd1* and *Hd3a*, respectively. The *PHOTOPERIOD SENSITIVITY5* (*SE5*) [37] gene, which encodes a hemeoxygenase for phytochrome chromophore biosynthesis, is absolutely required for photoperiodic flowering in rice. The *Arabidopsis* orthologue *LONG HYPOCOTYL1* (*HY1*) is also required for full response to photoperiod in *Arabidopsis* [38]. In addition, the diurnal mRNA expression patterns of circadian clock genes — *LHY1* (or *CCA1*) of *Arabidopsis* and *OsLHY* of rice, and *GI* of *Arabidopsis* and *OsGI* of rice — are conserved [11•,15••]. The circadian-clock-regulated patterns of *CAB* gene expression in rice are similar to those in *Arabidopsis* [39], suggesting that the circadian clock system is conserved.

Recent detailed studies of the conserved flowering pathway have highlighted the molecular difference in how short- and long-day plants measure daylength (Figure 2; [15••,25••,40,41,42••,43•]). *CO* promotes flowering under long-day conditions, whereas *Hd1* promotes flowering under short-day conditions and inhibits it under long-day

Figure 2



Comparison of gene interactions in the photoperiodic pathways of rice and *Arabidopsis*. Rice genes that have been assigned on the basis of sequence information only are shown in black. Rice genes assigned on the basis of mRNA expression analysis are shown in blue. The other rice genes (shown in red) are assigned on the basis of genetic evidence. Light has multiple actions on photoperiodic flowering. It is not clear whether *PHYB* affects *FT* mRNA expression in *Arabidopsis*. The transduction pathway for the external light signal that induces *LFY* and *SOC1* mRNA expression under long days remains unknown. Circadian clock mutants have not yet been found for rice. Os, *Oryza sativa*; RFL, RICE FLO/LFY; ZTL, ZEITLUPE.

conditions. The expression both *CO* and *Hd1* mRNAs is mainly regulated by the circadian clock. It has recently been revealed that *Hd1* functions as a repressor (activator) of mRNA expression of rice *FT* orthologues in interaction with (without) light-stable phytochrome signaling [15••]. On the other hand, *CO* promotes the expression of *FT* mRNA only in interaction with light signaling mediated by *CRY2* (under white light) and *PHYA*, a light-unstable phytochrome, (under far-red-rich light) [42••]. These studies provide the first insight into the molecular nature of the external coincidence model proposed by Bünning [44] and refined by Pittendrigh and Minis [45]. According to this model, light has two roles in allowing plants to measure daylength. First, light is involved in the entrainment of the circadian clock, which results in diurnal *Hd1* and *CO* expression from dusk till dawn. Second, light modifies the activity of the transcription factors *Hd1* and *CO*. The difference between rice and *Arabidopsis* largely comes from the differential action of external light signals on Hd1/CO activity. This differential action may be caused by domain differences between Hd1 and CO. Alternatively, different photoreceptor functions may be involved in the two species. In *Arabidopsis*, the *CRY2* function for floral promotion requires *PHYB* action, whereas *PHYB* mainly inhibits flowering under short days [46,47]. Light-stable *PHYB* action may therefore control *FT* expression in both rice and *Arabidopsis*, although no effect of *FT* expression by *PHYB* has been reported in *Arabidopsis* [48].

Protein phosphorylation may also play a conserved role in photoperiodic flowering in rice and *Arabidopsis*. *Hd6*, another rice QTL, inhibits flowering under long days [49] and encodes the α subunit of casein kinase2 (CK2). Some lines of evidence indicate that *CK2* is also involved in photoperiod response in *Arabidopsis*. Overexpression of *CKB3* — a protein that interacts with the circadian clock component *CCA1*, which encodes an *Arabidopsis* CK2β — increases CK2 activity, shortens periods of circadian rhythms, and results in early flowering with a reduced photoperiod response [50,51]. These results indicate that CK2 affects floral transition in both rice and *Arabidopsis*, maybe partly through the regulation of the circadian clock. Antisense expression of the *Arabidopsis CK2α* gene *ATHCK2A1* (or of an orthologue of *Hd6*) reduced CK2 activity and affected the expression of three light-regulated genes but did not significantly affect flowering time [52]. Further analysis will clarify the function of CK2 in both rice and *Arabidopsis*. It is now clear that the photoperiodic flowering pathway is evolutionarily conserved between rice and *Arabidopsis*. Whether other pathways, such as those for vernalization and gibberellin signaling, are conserved in these two species is the next obvious question for molecular geneticists.

Impacts of flowering-time QTLs

The analysis of natural allelic variation has contributed significantly to the exploration of genes that are involved in determining flowering time in rice [53]. Three QTLs for flowering time have been identified by map-based cloning [22,25••,49]. This work relied on standard processes for map-based cloning to identify gene function, including the construction of a physical map, chromosome walking, the sequencing of candidate genomic regions, and genetic complementation analysis. However, these processes are too time-consuming and laborious to be used by many researchers for the molecular identification of genes, especially at QTLs. The chromosome-aligned genome sequence information released by the International Rice Genome Sequencing Project allows us to skip several of the steps in map-based cloning [54].

A wide range of natural allelic variation exists in flowering-time genes because flowering time is a trait that adapts to environmental conditions such as photoperiod and temperature. For example, the Columbia and Landsberg *erecta* ecotypes of *Arabidopsis*, which are popular among molecular biologists, contain different alleles of the *FLC* and *FRIGIDA* floral regulators [29]. The introgression of new, naturally occurring alleles has contributed to the molecular analysis of flowering-times genes [29]. In addition, QTL analyses have provided clues to differences among functional alleles. A novel allele at *CRY2* has been identified through the analysis of the tropical Cape Verde Islands ecotype of *Arabidopsis* [55••]. Functional variation of *Hd1* alleles among rice cultivars has also been suggested [22]. Furthermore, map-based cloning of the flowering-time QTL *Hd3a* revealed a difference between two functional alleles at this locus. Nucleotide polymorphisms in non-coding sequences, such as introns and the 3′-untranslated region, may be involved in controlling the level of *Hd3a* transcription [25••].

The two subspecies of *Oryza sativa* (*indica* and *japonica*) and their wild relatives can be crossed to produce fertile progeny [56]. This situation provides the potential to mine a wide range of alleles using a map-based strategy [57].

Association study based on linkage disequilibrium

The use of whole-genome sequence information makes it easier to find plant genes on whose biological functions we can speculate. An alternative genomic approach, association study, can be used to identify the biological function of the candidate genes. Association studies are based on the linkage disequilibrium (LD) of candidate genes, that is, on the observation of a certain combination of alleles at different loci at a higher frequency than is predicted in a natural population. This approach has been widely used to study the complex genetics of many human diseases [58]. Association studies were thought to be limited to use in the identification of genes in artificially and naturally modified plant populations.

For instance, extensive LD was thought to prevent fine-scale mapping in self-pollinated plant species such as *Arabidopsis* and rice. However, recent studies in maize and *Arabidopsis* have demonstrated that the level of LD decays rapidly, suggesting that association study can achieve a resolution that is sufficient to define candidate genomic regions [59••,60••]. Association studies have also been used to identify the functions of genes in maize [61••], *Arabidopsis* [60••,62,63], and *Brassica nigra* [64•,65]. Moreover, this approach enabled researchers to finely localize quantitative trait nucleotides in candidate flowering-time genes [61••,64•].

In general, the level of LD depends on several factors, such as random genetic drift, and natural and artificial selection in crop species. More extensive surveys of local and genome-wide LD in crop plants will therefore be required to avoid the generation of false positives. Nevertheless, association study will be an effective approach for the functional identification of genes (or the high-resolution mapping of QTLs). It will also be an effective way to assess the contribution of particular candidate genes or functionally identified genes to the phenotypic variation among traits, such as flowering time, in naturally occurring plant populations.

Conclusions: 'comparison' starts to tell on the plant kingdom

The completion of rice genome sequencing and the comparison of genomic sequences within and between rice and *Arabidopsis* are providing great insights into the diversity among flowering plants. In addition, molecular genetic approaches such as forward, reverse, and transgene-based genetics can verify and refine these insights, as highlighted for photoperiodic flowering in this review. Furthermore, a gene-targeting approach that uses homologous recombination has been established recently in rice [66••]. This may become a powerful tool for the molecular identification of natural variations, which are often attributed to subtle nucleotide differences between functional alleles. Genome projects on other model plants, such as *Lotus japonicus*, *Medicago sativa*, and *Physcomitrella patens*, are ongoing. These will introduce us to a whole new world of genetic information on the plant kingdom.

Acknowledgements

The analysis of rice heading date was supported by the Programme for Promotion of Basic Research Activities for Innovative Biosciences and the Ministry of Agriculture, Forestry and Fisheries of Japan.

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- • of special interest
- •• of outstanding interest

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This study used the whole-genome shotgun method to sequence the rice genome. The *indica* rice cultivar '*93-11*', the parent of the super-hybrid rice '*Liang-You-Pei-Jiu*', was selected for this large project. In total, around 120 000 contigs were registered in the public sequence databases. The assembled sequence covers 92.0% of the genome. Free access to the database is essential to allow plant scientists to use the sequence information for the benefit of everyone.

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This study also used the whole-genome shotgun method to sequence the rice genome. This project used the *japonica* cultivar 'Nipponbare', which is also being sequenced by the International Rice Genome Sequencing Project. The assembled sequence covers 93% of the genome. The resulting database, containing around 40 000 contigs in total, can be searched only by registration with the Torrey Mesa Research Institute.

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This is the first report to explain how the short-day plant rice recognizes daylength at the molecular level. The evidence presented supports the external coincidence model, one of the most popular models for photo-

periodism. Daylengths other than 24 hours, such as 36 h light/12 h dark, were used to evaluate flowering-time mutants of rice.

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