The F-Box Gene Family Is Expanded in Herbaceous Annual Plants Relative to Woody Perennial Plants[1][W][OA]

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F-box proteins are generally responsible for substrate recognition in the Skp1-Cullin-F-box complexes that are involved in protein degradation via the ubiquitin-26S proteasome pathway. In plants, F-box genes influence a variety of biological processes, such as leaf senescence, branching, self-incompatibility, and responses to biotic and abiotic stresses. The number of F-box genes in *Populus* (*Populus trichocarpa*; approximately 320) is less than half that found in Arabidopsis (*Arabidopsis thaliana*; approximately 660) or *Oryza* (*Oryza sativa*; approximately 680), even though the total number of genes in *Populus* is equivalent to that in *Oryza* and 1.5 times that in Arabidopsis. We performed comparative genomics analysis between the woody perennial plant *Populus* and the herbaceous annual plants Arabidopsis and *Oryza* in order to explicate the functional implications of this large gene family. Our analyses reveal interspecific differences in genomic distribution, orthologous relationship, intron evolution, protein domain structure, and gene expression. The set of F-box genes shared by these species appear to be involved in core biological processes essential for plant growth and development; lineage-specific differences primarily occurred because of an expansion of the F-box genes via tandem duplications in Arabidopsis and *Oryza*. The number of F-box genes in the newly sequenced woody species *Vitis* (*Vitis vinifera*; 156) and *Carica* (*Carica papaya*; 139) is similar to that in *Populus*, supporting the hypothesis that the F-box gene family is expanded in herbaceous annual plants relative to woody perennial plants. This study provides insights into the relationship between the structure and composition of the F-box gene family in herbaceous and woody species and their associated developmental and physiological features.

The ubiquitin-proteasome-dependent pathway is one of the most elaborate protein degradation systems known. Ubiquitin and ubiquitin-like proteins are important in several cellular processes, including targeted protein degradation. Ubiquitination of proteins is commonly carried out by the E3-ubiquitin protein ligase complex specified through an isopeptide linkage between target protein (E3 bound) and ubiquitin (E2 bound). E3 ligases occur in monomeric or multimeric complexes (Mazzucotelli et al., 2006). A well-characterized multiple-subunit E3 ligase in plants is the Skp1-Cullin-F-box (SCF) protein complex (Kipreos and Pagano, 2000; Jin et al., 2005). The multiple steps required for protein ubiquitination, specificity, and deubiquitination are subject to control at many levels. SCF complexes are known to be regulated by the action of the COP9 signalosome, RUB, CAND1, microRNA, and transcriptional/posttranscriptional modification of various component complexes (Chang and Schwechheimer, 2004; Jones-Rhoades et al., 2006).

The distinguishing 50-amino acid F-box domain is a protein motif that functions as a site of protein-protein interaction (Kipreos and Pagano, 2000). F-box proteins are the substrate-recognition components of SCF ubiquitin-protein ligases. In plants, F-box proteins influence leaf senescence and branching (Woo et al., 2001; Stirnberg et al., 2007), flowering (Durfee et al., 2003; Imaizumi et al., 2005), circadian rhythms (Han et al., 2004; Kevei et al., 2006), self-incompatibility (Qiao et al., 2004; Sijacic et al., 2004; Wang et al., 2004; Takayama and Isogai, 2005), phytochrome signaling (Dieterle et al., 2001), and responses to plant growth regulators (abscisic acid, auxin, ethylene, and GA; Dill et al., 2004; Lai et al., 2004; Badescu and Napier, 2006; Binder et al., 2007) and abiotic (Calderon-Villalobos et al., 2007) and biotic (Kim and Delaney, 2002) factors.

Given the diverse set of developmental traits that F-box proteins are known to influence, it could be argued that long-lived woody plants would require a more abundant or elaborate system of protein degradation, when compared with short-lived herbaceous plants. That is, developmental changes in long-lived woody plants associated with juvenile versus mature, vegetative versus reproductive, and dormant versus nondormant states would lead to a more abundant set of F-box proteins. An alternative hypothesis may be that short-lived herbaceous plants would require a more strict, coordinated control of ontology in order to successfully complete development over a brief period

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of time. As such, short-lived plants would contain a more diverse set of gene regulation mechanisms, including ubiquitin-proteasome-dependent protein degradation, than would long-lived plants. In fact, the F-box gene number is twice as prevalent in the herbaceous annuals Arabidopsis (*Arabidopsis thaliana*) and *Oryza* (*Oryza sativa*) than it is in the perennial *Populus* (*Populus trichocarpa*; approximately 620 and approximately 690 versus approximately 300, respectively), even though the number of genes in the *Populus* genome (45,555) is equivalent to that in the *Oryza* genome (42,653) and 1.5 times that in the Arabidopsis genome (27,000; Haas et al., 2003; Tuskan et al., 2006; Ouyang et al., 2007). To illuminate the functional and comparative consequences of the aforementioned observation, we compared F-box-containing genes in Arabidopsis, *Populus*, and *Oryza* by analysis of phylogenetic relationships, protein domains, gene expression patterns, gene duplication, and intron evolution.

RESULTS

Genome-Wide Identification of F-Box Genes

A HMMER search of a customized database containing the annotated proteins of Arabidopsis (The Arabidopsis Information Resource [TAIR] release 7), *Oryza* (The Institute for Genomic Research [TIGR] release 5), and *Populus* (U.S. Department of Energy Joint Genome Institute [JGI] release 1.1) using the Pfam HMM profile built from 510 representative seed F-box proteins of diverse organisms, including animals and plants, identified 656 Arabidopsis, 678 *Oryza*, and 320 *Populus* predicted proteins (Supplemental Table S1).

Table I. *Distribution of F-box genes in the Populus genome*

Chromosome	Physical Length	Observed No. of F-Box Genes	Expected No. of F-Box Genes	Distribution Test[a]
	Mb			
LG I	32.16	36	30	0.137
LG II	23.44	25	22	0.235
LG III	17.45	13	17	0.235
LG IV	15.08	10	14	0.159
LG V	17.04	19	16	0.196
LG VI	17.68	19	17	0.242
LG VII	11.90	12	11	0.340
LG VIII	15.43	15	15	0.391
LG IX	12.41	12	12	0.395
LG X	19.21	15	18	0.273
LG XI	13.17	17	13	0.082
LG XII	13.03	13	12	0.353
LG XIII	11.50	13	11	0.207
LG XIV	13.68	13	13	0.421
LG XV	10.19	12	10	0.176
LG XVI	12.82	9	12	0.231
LG XVII	5.44	2	5	0.112
LG XVIII	12.44	12	12	0.398
LG XIX	10.23	2	10	0.004

[a]Note, for distribution test $P(m_{ij} < \lambda_{ij}) \leq \alpha$ or $P(m_{ij} > \lambda_{ij}) < \alpha$, a Poisson distribution was used to determine the significance of the F-box gene distribution in the *Populus* genome.

Table II. *Number of F-box genes resulting from segmental and tandem duplications in Arabidopsis, Oryza, and Populus genomes*

Segmental duplications are subchromosomal DNA segments with high identity, microsynteny, and shared total length.

Item	Arabidopsis	*Oryza*	*Populus*
Total no. of F-box genes	656	678	320
Tandem duplicates			
No. of F-box genes	236	291	73
Percentage of all F-box genes	36.0	42.9	22.8
Segmental duplicates			
No. of F-box genes	46	54	70
Percentage of all F-box genes	7.0	8.0	21.9

In *Populus*, F-box genes were found evenly distributed across all chromosomes in the genome, with the exception of chromosome XIX, on which the density of F-box genes is significantly lower in comparison with the other chromosomes (Table I). Of the 320 F-box genes in *Populus*, 74 (23% of the total) occur as tandem repeats, with the largest array containing four genes. An additional 22% of the total number of F-box genes in *Populus* was found within segmental duplications that arose as a result of the salicoid whole-genome duplication event experienced by all members of the genus (Tuskan et al., 2006). Moreover, eight F-box genes that are part of two tandem arrays occurred as the result of at least one paralogous duplication.

The number of F-box genes occurring as tandem repeats in Arabidopsis and *Oryza*, 236 (36% of the total) and 291 (43%), respectively, is higher than that in *Populus*, whereas the number of F-box genes occurring as segmental duplicates in Arabidopsis and *Oryza*, 46 (7%) and 54 (8%), respectively, is substantially lower than that in *Populus* (Table II). Interestingly, there are two tandem repeats in Arabidopsis that occur as homologs in *Populus* and two additional tandem repeats that are homologous in all three species. Each of these arrays contains four genes in tandem order. This suggests that these genomic segments were present in the last shared common ancestor and that this gene family has experienced tandem expansions over the past 120 million years. Finally, in 9% and 18% of the duplications in Arabidopsis and *Oryza*, respectively, the F-box motifs were missing in one copy of the two duplicates (data not shown), implying that gene diversification and domain loss has occurred after gene duplication.

Phylogeny and Orthologous Clustering

To examine the relationship among the 1,654 analyzed F-box proteins in Arabidopsis, *Oryza*, and *Populus*, a gene-based phylogenetic tree was created using full-length protein sequences (Fig. 1). The F-box proteins were divided into 50 distinct phylogenetic

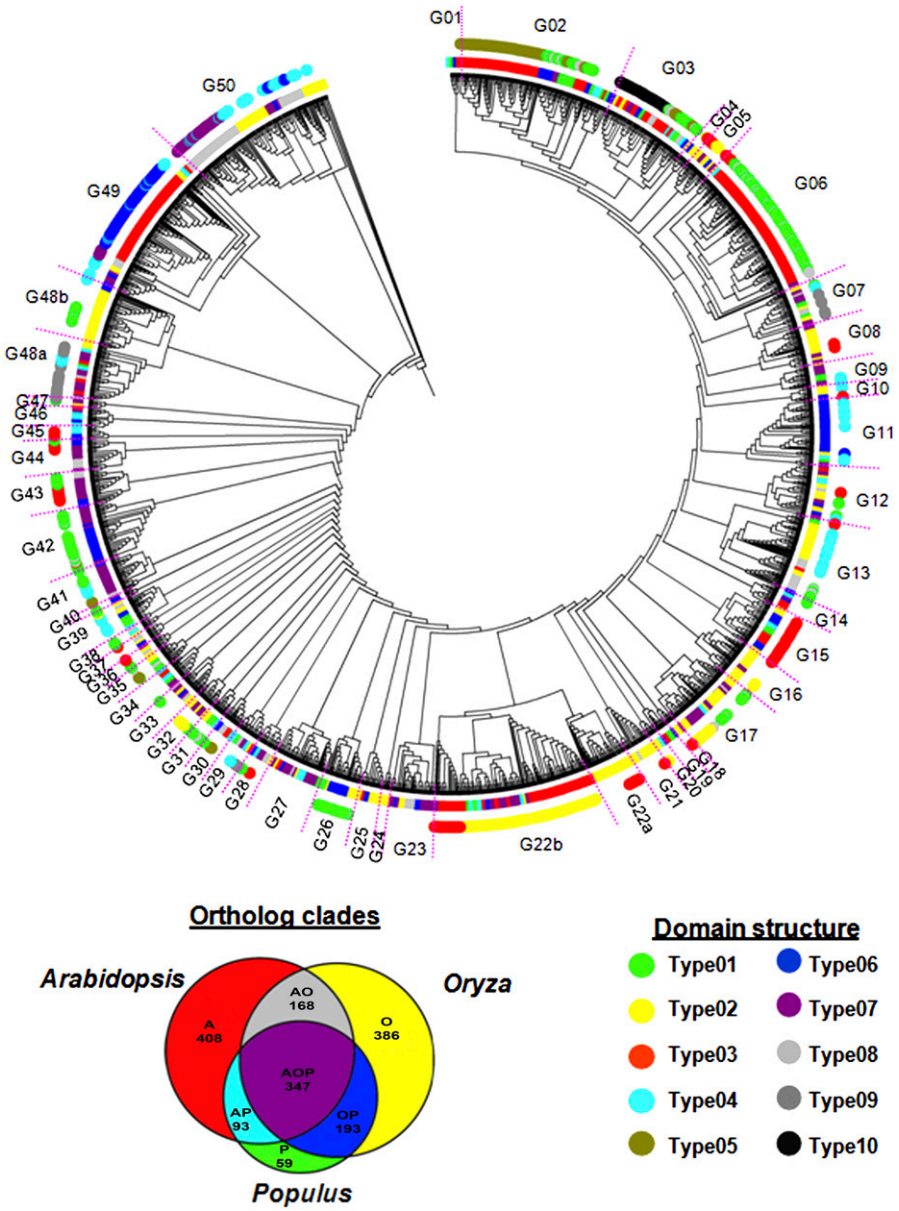

Figure 1. A phylogeny created with the full-length F-box protein sequences in Arabidopsis, *Oryza*, and *Populus*. The numbers in the Venn diagram represent the number of genes per ortholog clade. Domain structure types are defined in Figure 3. The interior colored ring corresponds to the distribution of orthologous clades; the colors are as depicted in the Venn diagram. The outer colored ring corresponds to the domain structure distribution.

groups (designated G01–G50) based on manual delineation of the phylogenetic tree.

To identify orthologous clades (i.e. genes originating from a single ancestral gene in the last common ancestor of the compared genomes) among the F-box proteins in the three plant species, a reconciled phylogenetic tree (Supplemental Fig. S1) was constructed by combining the gene tree (Fig. 1) and the species tree (i.e. [[Arabidopsis, *Populus*], *Oryza*]). The F-box proteins were then divided into seven clades: AOP (Arabidopsis-*Oryza*-*Populus*), AO (Arabidopsis-*Oryza*), OP (*Oryza*-*Populus*), AP (Arabidopsis-*Populus*), A (Arabidopsis specific), O (*Oryza* specific), and P (*Populus* specific). The AOP clade contains genes having orthologs in Arabidopsis, *Oryza*, and *Populus*; the AP clade contains genes having orthologs in Arabidopsis and *Populus*, et cetera. It is noteworthy that the number of genes in the A clade is equivalent to that in the O clade and about six times that in the P clade (Fig. 2A), suggesting lineage-specific F-box gene expansions in the annual herbaceous species.

The F-box genes in the A clade occurred more often than expected by chance alone in phylogenetic groups G02, G06, G22b, and G49 ($P \leq 0.001$; Table III; Fig. 1), indicating that these groups of genes may have experienced expansion in Arabidopsis. Examples of well-characterized genes of the A clade include *CEGENDUO* and *SON1* in group G06 and *FBX7* in group G22b (Supplemental Table S2). F-box genes in the P clade occurred more often than expected by chance alone in the phylogenetic groups G02, G27, G35, and G39 ($P \leq 0.001$). We hypothesize that these groups of genes may

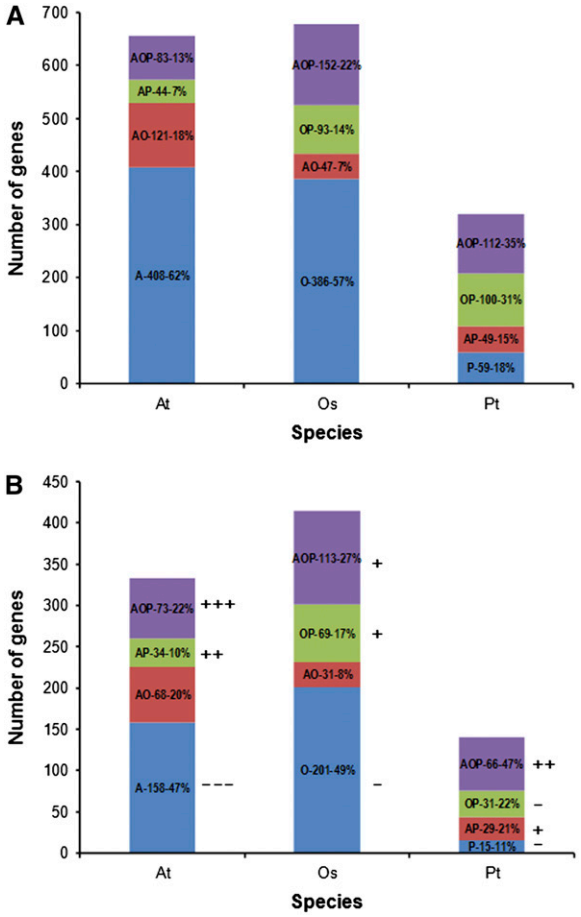

Figure 2. Ortholog clades of F-box genes in Arabidopsis, *Oryza*, and *Populus*. A, All F-box genes including expressed and unexpressed genes. B, F-box genes with expression evidence. +, ++, and +++ indicate that genes with expression evidence are overrepresented at $P \leq 0.05$, 0.01, and 0.001, respectively, − and −−− indicate underrepresented genes at $P \leq 0.05$ and 0.001, respectively, as compared with all F-box genes. Data label: AOP-83-13% represents 83 F-box genes (13% of the total F-box genes in Arabidopsis) found in the AOP clade.

be uniquely related to perennial or woody habit. The AOP clade is overrepresented in the phylogenetic groups G09, G17, G23, G27, G41, G43, G44, and G48a ($P \leq 0.001$), indicating that these groups of genes, shared by the three plant species, may be involved in basic biological processes required for general plant growth and development. Some well-characterized genes of the AOP clade associated with common plant growth and development include *ARABIDILLO1* and *ARABIDILLO2* in group G12 and *AtFBP7* in group G13 (Supplemental Table S2).

Homologs in Other Herbaceous Monocot, Herbaceous Eudicot, and Woody Eudicot Species

To test the validity of the hypotheses stated above, we investigated the homology of the F-box proteins in Arabidopsis, *Populus*, and *Oryza* with genes in other plants by BLAST search against transcript assemblies of 193 plant species (Childs et al., 2007; Supplemental Table S3). Among all herbaceous monocot, herbaceous eudicot, and woody eudicot EST data sets, the homologs of clade A or AP were significantly overrepresented in both sets of eudicots and underrepresented in herbaceous monocots; the homologs of clade O were overrepresented in the herbaceous monocot data set but underrepresented in all eudicots; the homologs of clade P were overrepresented in woody eudicots, including *Vitis* and *Eucalyptus*, and underrepresented in herbaceous monocots; and the homologs of clade AO were overrepresented in herbaceous eudicots but underrepresented in woody eudicots (Table IV). These data clearly support the ortholog classification based on the phylogenetic tree and indicate that the majority of the genes in the species-specific clades (i.e. A, O, or P) share genomic/genic features with other monocots versus eudicots and/or herbaceous versus woody species.

Protein Motif Structure

InterProScan identified more than 90 types of protein motif structures in the 1,654 studied F-box proteins (Supplemental Table S4). Thirty-five percent of the F-box proteins (579 of 1,654) contained only a single motif (i.e. the F-box domain). Among the remaining 1,075 F-box proteins, 793 proteins (approximately 74%) contained one or more of the 10 most common protein motif structures (Fig. 3). Protein motif structure types 1, 5, and 6, containing F-box-associated domains, Leu-rich repeat 2 domains, and FBD domains, respectively, occurred more often than expected by chance alone in genes in the A clade ($P \leq 0.001$). Protein motif structure types 2 and 9, containing Kelch-related and Leu-rich repeat domains, respectively, occurred more often than expected in genes in the AOP clade ($P \leq 0.001$), indicating that these motifs may be associated with the basic biological processes shared by all three species.

Intron-Exon Structure

To contrast gene structures among the examined species, we compared the intron composition of the F-box genes by dividing gene structures into four bins: intronless, one intron, two introns, and three or more introns per gene. In general, the F-box genes in Arabidopsis, *Oryza*, and *Populus* contain more intronless genes and fewer three-or-more-intron genes than expected by chance alone when compared with all other genes in each examined genome ($P \leq 0.0001$). Moreover, F-box genes in the A, P, and AP clades contain more intronless gene structures ($P \leq 0.001$), and the AOP clade contains more genes with three or more introns ($P \leq 0.001$), than expected by chance alone when compared with all other F-box genes (Table V). Carmel et al. (2007) suggested that the loss of introns is associated with recent evolutionary ex-

Table III. *Overrepresentation or underrepresentation of ortholog clades in each phylogenetic group, as compared with the average distribution across all 1,654 F-box genes*

P values were calculated using the cumulative Poisson distribution. The "Genes" columns represent the observed and expected (in parentheses) numbers of genes.

Group[a]	A		O		P		AO		AP		OP		AOP		Total
	Genes	*P*	Genes	*P*	Genes	*P*	Genes	*P*	Genes	*P*	Genes	*P*	Genes	*P*	
G02	79 (29)	0.E+00[b]	0 (28)	1.E-12	14 (4)	4.E-05[b]	0 (12)	6.E-06[c]	6 (7)	0.5054	14 (14)	0.4051	5 (25)	2.E-06[c]	118
G03	19 (13)	0.0447	4 (12)	0.0059	3 (2)	0.1237	8 (5)	0.0960	6 (3)	0.0325	8 (6)	0.1724	5 (11)	0.0349	53
G05	0 (4)	0.0118	12 (4)	0.0004[b]	0 (1)	0.5262	0 (2)	0.1607	0 (1)	0.3635	2 (2)	0.6495	4 (4)	0.3276	18
G06	116 (30)	0.E+00[b]	1 (28)	1.E-11	0 (4)	0.0129	0 (12)	4.E-06[c]	2 (7)	0.0329	0 (14)	7.E-07[c]	3 (26)	2.E-08[c]	122
G07	0 (6)	0.0016	5 (6)	0.4349	3 (1)	0.0149	5 (3)	0.0521	0 (1)	0.2318	0 (3)	0.0481	13 (5)	0.0016	26
G08	0 (8)	0.0004[c]	20 (7)	4.E-05[b]	0 (1)	0.3193	0 (3)	0.0388	0 (2)	0.1654	0 (4)	0.0239	12 (7)	0.0202	32
G09	0 (4)	0.0193	1 (4)	0.1131	4 (1)	0.9997	0 (2)	0.1969	0 (1)	0.4067	0 (2)	0.1546	11 (3)	0.0002[b]	16
G10	0 (2)	0.0849	6 (2)	0.0101	0 (0)	0.7000	0 (1)	0.3621	0 (1)	0.5699	0 (1)	0.3113	4 (2)	0.0619	10
G11	0 (14)	1.E-06[c]	2 (13)	0.0002[c]	1 (2)	0.4067	0 (6)	0.0034	3 (3)	0.6139	50 (7)	0.E+00[b]	0 (12)	8.E-06[c]	56
G12	1 (9)	0.0017	11 (8)	0.1244	0 (1)	0.2869	2 (4)	0.3108	4 (2)	0.0498	3 (4)	0.4172	14 (7)	0.0086	35
G13	1 (15)	5.E-06[c]	34 (14)	2.E-06[b]	2 (2)	0.6292	20 (6)	2.E-06[b]	0 (3)	0.0324	0 (7)	0.0008[c]	4 (13)	0.0043	61
G14	2 (3)	0.4903	0 (3)	0.0768	0 (0)	0.6754	0 (1)	0.3272	2 (1)	0.0250	4 (1)	0.0101	3 (2)	0.2022	11
G15	7 (8)	0.4004	6 (8)	0.3214	4 (1)	0.0081	0 (3)	0.0316	4 (2)	0.0450	13 (4)	0.0001[b]	0 (7)	0.0008[c]	34
G16	0 (7)	0.0008[c]	25 (7)	1.E-08[b]	0 (1)	0.3554	0 (3)	0.0526	0 (2)	0.1958	0 (3)	0.0339	4 (6)	0.2740	29
G17	0 (14)	1.E-06[c]	26 (13)	0.0004[b]	0 (2)	0.1406	1 (6)	0.0247	3 (3)	0.6265	0 (6)	0.0016	25 (12)	0.0002[b]	55
G21	0 (5)	0.0072	18 (5)	5.E-07[b]	0 (1)	0.4900	2 (2)	0.6682	0 (1)	0.3248	0 (2)	0.0969	0 (4)	0.0151	20
G22a	0 (10)	0.0001[c]	39 (9)	3.E-14[b]	0 (1)	0.2488	0 (4)	0.0190	0 (2)	0.1116	0 (5)	0.0106	0 (8)	0.0003[c]	39
G22b	84 (30)	0.E+00[b]	1 (29)	1.E-11	4 (4)	0.5536	0 (12)	4.E-06[c]	8 (7)	0.2600	3 (14)	0.0004[c]	23 (26)	0.3346	123
G23	0 (9)	0.0002[c]	3 (8)	0.0378	0 (1)	0.2869	8 (4)	0.0108	0 (2)	0.1397	5 (4)	0.2281	19 (7)	0.0001[b]	35
G25	1 (4)	0.0642	14 (4)	3.E-05[b]	0 (1)	0.5262	0 (2)	0.1607	0 (1)	0.3635	3 (2)	0.1614	0 (4)	0.0229	18
G26	0 (7)	0.0008[c]	12 (7)	0.0214	0 (1)	0.3554	0 (3)	0.0526	0 (2)	0.1958	17 (3)	2.E-08[b]	0 (6)	0.0023	29
G27	1 (10)	0.0007[c]	2 (9)	0.0057	7 (1)	0.0001[b]	1 (4)	0.0945	5 (2)	0.0246	1 (5)	0.0586	22 (8)	2.E-05[b]	39
G28	0 (4)	0.0151	2 (4)	0.2429	0 (1)	0.5453	4 (2)	0.0313	2 (1)	0.0724	0 (2)	0.1376	9 (4)	0.0038	17
G29	1 (3)	0.1703	1 (3)	0.1941	1 (0)	0.0794	2 (1)	0.1476	2 (1)	0.0380	2 (2)	0.1954	4 (3)	0.1412	13
G30	3 (3)	0.6010	0 (3)	0.0481	0 (0)	0.6289	4 (1)	0.0113	6 (1)	1.E-05[b]	0 (2)	0.2194	0 (3)	0.0654	13
G32	0 (4)	0.0193	7 (4)	0.0368	0 (1)	0.5651	0 (2)	0.1969	0 (1)	0.4067	0 (2)	0.1546	9 (3)	0.0025	16
G33	0 (4)	0.0247	9 (4)	0.0033	0 (1)	0.5856	2 (2)	0.1971	0 (1)	0.4302	1 (2)	0.4778	3 (3)	0.6144	15
G34	0 (3)	0.0405	0 (3)	0.0481	1 (0)	0.0794	1 (1)	0.6196	3 (1)	0.0067	0 (2)	0.2194	8 (3)	0.0020	13
G35	3 (2)	0.2353	0 (2)	0.0969	3 (0)	0.0005[b]	2 (1)	0.0832	2 (1)	0.0196	0 (1)	0.3113	0 (2)	0.1227	10
G39	0 (4)	0.0151	2 (4)	0.2429	5 (1)	4.E-05[b]	3 (2)	0.0972	4 (1)	0.0030	3 (2)	0.1399	0 (4)	0.0283	17
G41	0 (8)	0.0005[c]	0 (7)	0.0007[c]	0 (1)	0.3309	2 (3)	0.3907	0 (2)	0.1750	3 (4)	0.5115	26 (7)	2.E-09[b]	31
G42	0 (10)	0.0001[c]	0 (9)	0.0001[c]	0 (1)	0.2488	0 (4)	0.0190	0 (2)	0.1116	23 (5)	1.E-10[b]	16 (8)	0.0046	39
G43	0 (7)	0.0008[c]	0 (7)	0.0012	0 (1)	0.3554	0 (3)	0.0526	0 (2)	0.1958	4 (3)	0.2528	25 (6)	2.E-09[b]	29
G44	0 (6)	0.0027	0 (6)	0.0037	0 (1)	0.4248	9 (2)	0.0002[b]	0 (1)	0.2594	0 (3)	0.0608	15 (5)	0.0001[b]	24
G46	0 (4)	0.0193	0 (4)	0.0239	0 (1)	0.5651	0 (2)	0.1969	14 (1)	6.E-14[b]	2 (2)	0.2874	0 (3)	0.0348	16
G48a	3 (10)	0.0136	0 (9)	0.0001[c]	1 (1)	0.5949	3 (4)	0.4411	5 (2)	0.0246	0 (5)	0.0106	27 (8)	5.E-08[b]	39
G48b	0 (13)	2.E-06[c]	53 (12)	0.E+00[b]	0 (2)	2.E-01	0 (5)	0.0046	0 (3)	0.0508	0 (6)	0.0021	0 (11)	1.E-05[c]	53
G49	85 (29)	0.E+00[b]	7 (28)	3.E-06[c]	0 (4)	0.0149	6 (12)	0.0462	4 (7)	0.2090	8 (14)	0.0694	8 (25)	0.0001[c]	118
G50	0 (32)	2.E-14[c]	40 (30)	0.0306	0 (5)	1.E-02	76 (13)	0.E+00[b]	0 (7)	0.0007[c]	2 (15)	4.E-05[c]	10 (27)	0.0002[c]	128

[a]Phylogenetic groups are depicted in Figure 1. Groups with fewer than 10 genes are not shown. [b]Overrepresentation of ortholog clades in each phylogenetic group. [c]Underrepresentation of ortholog clades in each phylogenetic group.

pansion in large gene families. Our data support their conclusion and suggest that recent lineage-specific expansion of F-box gene family members has occurred among the three examined species.

Gene Expression and Predicted Function

In Arabidopsis, *Oryza*, and *Populus*, 333 (51% of the total), 414 (61%), and 141 (44%), respectively, of the predicted F-box genes have expression evidence (i.e. ESTs and/or full-length cDNA data). Among the genes with expression evidence, the A, O, and P clades are significantly underrepresented and the AOP clade is overrepresented when compared with all genes (Fig. 2B), demonstrating that genes common to all three species are more frequently represented in such databases and genes uniquely found in Arabidopsis, *Populus*, or *Oryza* are less common in publicly available gene expression databases. This observation could be due to sampling error within the tested libraries or differences in the expression of recently evolved lineage-specific members of the F-box family, where lineage-specific genes may be infrequently expressed and as of yet uncatalogued.

In addition to the F-box-containing genes, there are several other genes associated with the SCF complex, including CAND1, COP9, Cul1, E1, E2, RBX1, ROC1, RUB1/2, and SKP1/ASK1/ASK2 (Lechner et al., 2006). A Spearman's rank correlation indicated that the 320 *Populus* F-box genes and 146 SCF-associated genes are expressed in a coordinated manner across nine different *Populus* tissue types ($r = 0.97$, $P \leq 0.0001$; Fig. 4). Similarly, expression patterns for F-box and SCF complex-related genes in Arabidopsis in both the developmental and environmental data sets are correlated ($r = 0.79$, $P \leq 0.002$). These data indicate that there is a transcriptional relationship between F-box genes and their associated protein complexes in Arabidopsis and *Populus*. In addition to the F-box members of the SCF complex, there are alternative

Table IV. *Overrepresentation or underrepresentation in herbaceous monocot, herbaceous eudicot, or woody eudicot species of homologs obtained by a tBLASTn search of the plant transcript assemblies (Childs et al., 2007; Supplemental Table S3) using the F-box proteins of clades A, O, P, AO, AP, or OP, as compared with the AOP clade, with an e-value cutoff of 1E^{-30}*

P value was calculated using the cumulative Poisson distribution.

Ortholog Clade of Query	Measure	No. of Observed and Expected F-Box Genes by Category		
		Herbaceous Monocot	Herbaceous Eudicot	Woody Eudicot
A	Observed	1,044	2,564	1,179
	Expected	1,296	2,426	1,065
	P	1.9E-13[c]	2.6E-03[b]	2.8E-04[b]
O	Observed	4,869	1,288	642
	Expected	1,841	3,445	1,513
	P	0.0E+00[b]	0.0E+00[c]	0.0E+00[c]
P	Observed	341	993	500
	Expected	497	929	408
	P	5.7E-14[c]	1.8E-02	4.7E-06[b]
AO	Observed	1,760	3,516	1,078
	Expected	1,721	3,220	1,414
	P	1.7E-01	0.0E+00[b]	0.0E+00[c]
AP	Observed	792	2,516	1,320
	Expected	1,253	2,345	1,030
	P	0.0E+00[c]	2.1E-04[b]	0.0E+00[b]
OP	Observed	1,954	3,116	1,380
	Expected	1,747	3,268	1,435
	P	0.0E+00[b]	3.7E-03[c]	7.5E-02
AOP[a]	Observed	8,449	15,810	6,941

[a]The AOP clade was used as a reference for comparison and contains F-box genes that are homologous by clade that were initially identified in Arabidopsis, *Oryza*, and *Populus*. [b]Overrepresentation in herbaceous monocot, herbaceous eudicot, or woody eudicot species of homologs obtained by a tBLASTn search of the plant transcript assemblies. [c]Underrepresentation in herbaceous monocot, herbaceous eudicot, or woody eudicot species of homologs obtained by a tBLASTn search of the plant transcript assemblies.

members of the substrate-specific E3 ligase pathways, include HECT, RING, and U-box proteins. Both Arabidopsis and *Populus* have significantly more RING proteins than *Oryza*, and *Oryza* has more CUL3-BTB3 proteins than Arabidopsis and *Populus* (Table VI), suggesting that the large differences in numbers of F-box genes in Arabidopsis versus *Populus* or *Oryza* versus *Populus* are not being compensated for by alternative ubiquitination pathways.

A Gene Ontology (GO) analysis was performed to further characterize the predicted functions of the F-box proteins. Essential biological processes, including signal transduction, flower development, regulation of circadian rhythm, lateral root formation, and actin filament-based processes, occurred significantly more frequently in the AOP clade, whereas the genes associated with responses to biotic stresses were significantly enriched in the A clade (Table VII), suggesting that (1) Arabidopsis, *Oryza*, and *Populus* share some essential biological pathways mediated by F-box proteins and (2) the lineage-specific expansion of F-box genes in Arabidopsis.

Vitis and *Carica*

A phylogenetic analysis was performed on the F-box genes in *Populus*, *Vitis* (*Vitis vinifera*), and *Carica* (*Carica papaya*; Supplemental Table S5; Supplemental Fig. S2). Based on the previously described HMMER search criteria, we identified 156 and 139 F-box genes in the newly sequenced *Vitis* (Jaillon et al., 2007) and *Carica* genomes, respectively. The *Populus* genome has experienced a whole-genome duplication event (Tuskan et al., 2006) that is not shared by *Vitis* or *Carica*; thus, the 320 F-box genes are in agreement with the detected F-box genes in *Vitis* and *Carica*. Interestingly, among the *Populus* F-box genes found in the AOP clade, 54% had no homologs in the *Vitis* and *Carica* genomes (Fig. 5). In contrast, among the *Populus* F-box genes found uniquely in the P clade, 75% had no homologs in the *Vitis* and *Carica* genomes. These data clearly show that even though *Populus* experienced a whole-genome duplication that was not shared by *Vitis* or *Carica*, there are significantly fewer F-box genes in all woody perennials compared with Arabidopsis and *Oryza*. These results support our hypothesis that woody perennial plants have fewer F-box genes relative to herbaceous annuals.

DISCUSSION

F-box proteins represent a large gene family in most eukaryotic organisms and appear to be underrepre-

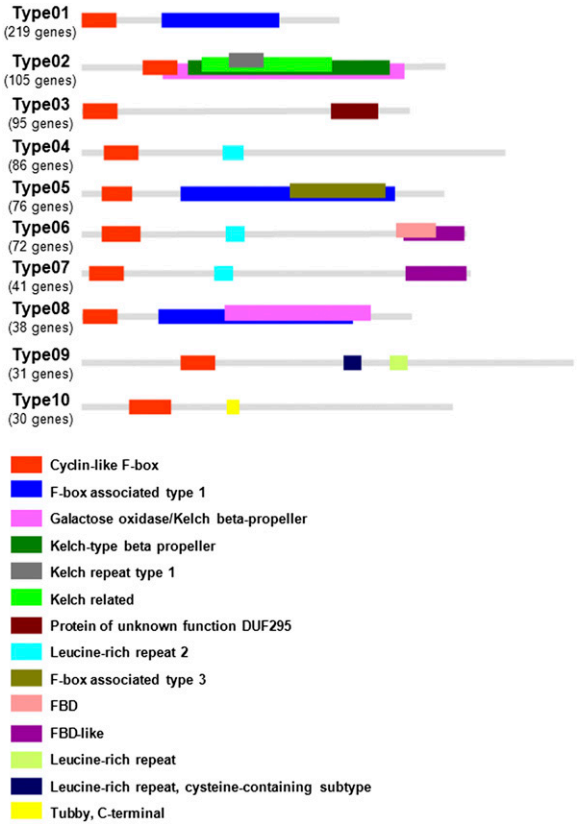

Figure 3. The 10 most common domain structures found in F-box proteins of Arabidopsis, *Populus*, and *Oryza*. The total number of genes associated with each domain structure is shown in parentheses.

sented in *Populus*, *Vitis*, and *Carica* relative to Arabidopsis and *Oryza*. This study has explored (1) the extent of lineage/species-specific differential distribution of F-box genes among various subgroups within this gene family and (2) the functional implications of differential representation toward cellular and biological processes. For example, the genes involved in actin filament-based processes were found uniquely within the AOP clade. Alternatively, the up-regulated expression of 38 A clade self-incompatibility genes, mainly in pollen, points toward lineage-specific expansion that has played an important role in flower development and successful reproduction in Arabidopsis (Supplemental Table S6). Self-incompatibility genes were not found in the dioecious *Populus* (Yin et al., 2008).

In addition to the role that the F-box proteins play in mediating innate signals for developmental transition, another aspect for protein turnover may be related to rapid responses to external signals such as environmental cues and stressors. The presence of a much larger F-box gene family in plants (i.e. Arabidopsis, *Oryza*, *Populus*, *Vitis*, and *Carica*) when compared with less than 100 genes in animals (i.e. human, mouse, and *Drosophila*) suggests a predominant role for members of this gene family in the management of responses to environmental signals in immobile organisms.

Although *Populus* has half as many F-box genes as Arabidopsis, our results also confirm that certain F-box genes associated with developmental roles in organ boundary determination (e.g. *HAWAIIAN SKIRT*), floral organ development (e.g. *UFO*), and photoperiod and plant growth response signaling (e.g. vernalization-response [*FKF1*], circadian rhythm signaling [*ZTL*], phytochrome A-specific light signaling [*EID1*], ethylene perception [*EBF1*], GA signaling [*SLEEPY1*], and auxin signaling [*TIR1*]) have expanded in *Populus* relative to *Oryza* and Arabidopsis (Supplemental Fig. S3).

Yet another distinctive feature of the F-box gene family is the relatively high proportion of intronless genes. Carmel et al. (2007) suggested that high intron density was reached in the early evolutionary history of plants and that the last common ancestor of multicellular life forms harbored approximately 3.4 introns per kilobase, a greater intron density than in most of the extant fungi and in some animals. A recent report also implies that rates of intron creation were higher during earlier periods of plant evolution (Roy and Penny, 2007). Our results support these hypotheses in that the A, P, and AP clades are overrepresented by intronless gene structures and the AOP clade is overrepresented by genes with three or more introns. From this perspective, we conjecture that the F-box gene

Table V. *Overrepresentation or underrepresentation of intron numbers per gene in each ortholog clade, as compared with all 1,654 F-box genes*

P values were calculated using the cumulative Poisson distribution. The "Genes" columns represent the observed and expected (in parentheses) numbers of genes.

Ortholog Clade[a]	Intronless		One Intron		Two Introns		Three or More Introns	
	Genes	*P*	Genes	*P*	Genes	*P*	Genes	*P*
A	210 (145)	2.E-07[b]	76 (86)	0.1443	54 (77)	0.0031[c]	68 (99)	0.0005[c]
O	133 (137)	0.3878	87 (82)	0.2563	76 (73)	0.3472	90 (94)	0.3628
P	41 (21)	3.E-05[b]	10 (12)	0.2985	4 (11)	0.0132	4 (14)	0.0014[c]
AO	15 (60)	6.E-12[c]	36 (36)	0.4260	68 (32)	9.E-09[b]	49 (41)	0.0932
AP	47 (33)	0.0084[b]	18 (20)	0.4089	17 (18)	0.5012	11 (23)	0.0053[c]
OP	47 (68)	0.0038[c]	48 (41)	0.1172	29 (37)	0.1165	69 (47)	0.0010[b]
AOP	94 (123)	0.0037[c]	75 (73)	0.3974	66 (66)	0.4612	112 (85)	0.0018[b]

[a]Ortholog clades are depicted in Figure 1. [b]Overrepresentation of intron numbers per gene in each ortholog clade. [c]Underrepresentation of intron numbers per gene in each ortholog clade.

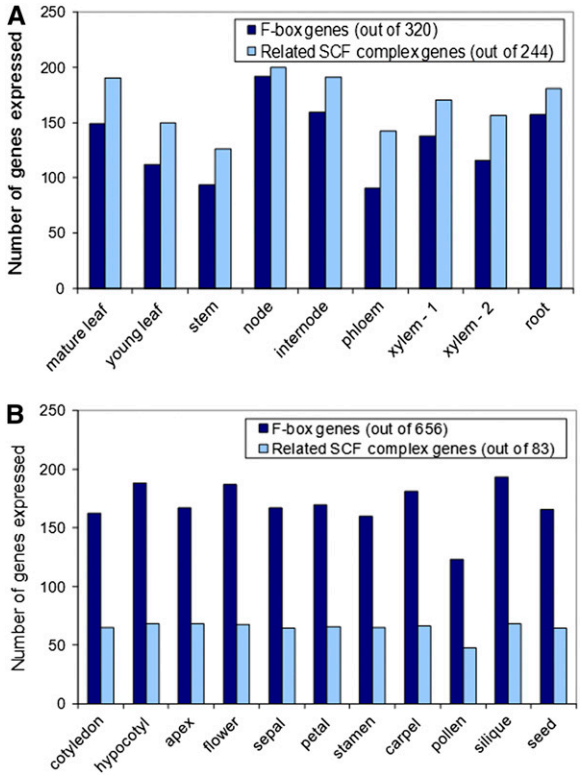

Figure 4. The number of F-box and SCF complex-related genes expressed in *Populus* (A) and Arabidopsis (B) whole-genome microarray data. A Spearman's rank correlation with $r = 0.97$ and $r = 0.79$, respectively, indicates that F-box and SCF complex-related genes appear to be expressed in a similar pattern across the tissue types included in the analysis ($P \leq 0.01$).

family members in Arabidopsis, *Oryza*, and *Populus* have experienced expansion since they last shared a common ancestor.

The SCF ubiquitin-proteasome-dependent pathway is one of the most elaborate and common protein-degradation systems. There are alternative pathways to ubiquitination in plants (Jin et al., 2005). The single-subunit ubiquitination complex, HECT, is twice as common in *Populus*, which argues against the link between reduced F-box gene number and the extent of ubiquitination, although the HECT pathway is thought to be used less frequently in most organisms. Members of the RING and APC complexes are nearly twice as abundant in Arabidopsis and *Populus* as they are in *Oryza*, and members of the CUL3-BTB3 complex are nearly twice as abundant in *Oryza* as they are in Arabidopsis and *Populus*, suggesting that dicots and monocots utilize these pathways in a differential manner.

The relatively fewer F-box genes in *Populus* must be integral to biological processes in *Populus*. In Arabidopsis, functional redundancy among members of this large gene family appears to account for some of the expansion. *TIR1*, *AFB1*, *AFB2*, and *AFB3* quadruple mutants are reported to be viable (Dharmasiri et al., 2005). The smaller number of F-box genes in *Populus* suggests that in *Populus*, F-box proteins may have evolved to recognize multiple substrates (i.e. multifunctional/multiaffinity F-box proteins), contain fewer conserved pathways, and/or have alternative pathways that are functionally redundant. The recent expansion of the F-box gene family in Arabidopsis and *Oryza* compared with *Populus*, *Vitis*, and *Carica* may reflect a comparatively reduced need for ubiquitination-mediated protein turnover in long-lived perennial plants. However, because it is difficult to compare developmental stages between perennial and annual plants, we cannot conclusively determine the extent of the proteome that is ubiquitinated in *Populus* or *Vitis* relative to Arabidopsis or *Oryza* for any given ontogeny. Future proteomics investigations may shed light on the extent and prevalence of the ubiquitination pathway in *Populus*, and in particular on whether the SCF pathway is employed to a lesser extent in long-lived plants.

CONCLUSION

This study was undertaken to explore, through comparative bioinformatics, the qualitative and quantitative differences among the F-box genes present in three sequenced plant genomes. We further explored how the relative disparity of the F-box gene family in *Populus* may reflect the biology of this organism. Our results have shed light on several key differences in F-box gene family evolution between the three species, provided insights into the structure and composition of F-box gene family members in relation to distinguishing developmental and physiological features, and demonstrated that although the overall family size is smaller in *Populus*, certain subgroups containing genes with known roles in light response and plant

Table VI. *Number of substrate-specific E3 ligase genes in Arabidopsis, Oryza, and Populus*

Complex	Gene Family	Domain for InterProScan	Arabidopsis	*Oryza*	*Populus*
HECT	HECT	IPR000569 (HECT)	7	8	16
RING	RING	IPR001841 (zinc finger, RING type) or IPR013083 (zinc finger, RING/FYVE/PHD type)	477	259	459
U-box	U-box	IPR003613 (U-box)	53	60	84
APC	CDC20	IPR000002 (Cdc20/Fizzy)	8	5	9
CUL3-BTB3	BTB	IPR000210 (BTB/POZ-like) or IPR013069 (BTB/POZ) or IPR011333 (BTB/POZ fold)	72	138	85

Table VII. *GO term enrichment in F-box proteins*

GO Identifier	Biological Process	Percentage of Total Query Genes[b]	Percentage of Total Reference Genes[b]	Unadjusted *P*[c]	Adjusted *P*[c]
AOP versus non-AOP[a]					
GO:0007165	Signal transduction	3.98	0	$7E^{-06}$	0.0003
GO:0006355	Regulation of transcription	3.98	0	$7E^{-06}$	0.0003
GO:0006511	Ubiquitin-dependent protein catabolic process	21.24	9.24	$1E^{-05}$	0.0003
GO:0009908	Flower development	3.98	0.17	$5E^{-05}$	0.0009
GO:0043153	Entrainment of circadian clock by photoperiod	3.98	0.17	$5E^{-05}$	0.0009
GO:0042752	Regulation of circadian rhythm	3.98	0.17	$5E^{-05}$	0.0009
GO:0010114	Response to red light	3.98	0.17	$5E^{-05}$	0.0009
GO:0048589	Developmental growth	3.98	0.5	$7E^{-04}$	0.0050
GO:0045014	Negative regulation of transcription by Glc	3.98	0.5	$7E^{-04}$	0.0050
GO:0009733	Response to auxin stimulus	3.98	0.5	$7E^{-04}$	0.0050
GO:0002237	Response to molecule of bacterial origin	3.98	0.5	$7E^{-04}$	0.0050
GO:0010311	Lateral root formation	3.98	0.5	$7E^{-04}$	0.0050
GO:0031146	SCF-dependent proteasomal ubiquitin-dependent protein catabolic process	2.21	0	$1E^{-03}$	0.0097
GO:0030029	Actin filament-based process	1.77	0	$5E^{-03}$	0.0330
A versus non-A[a]					
GO:0009620	Response to fungus	5.76	0	$7E^{-08}$	$2.E^{-06}$
GO:0009617	Response to bacterium	5.76	0	$7E^{-08}$	$2.E^{-06}$
GO:0044267	Cellular protein metabolic process	4.71	0	$2E^{-06}$	$3.E^{-05}$
GO:0051707	Response to other organism	3.66	0	$3E^{-05}$	0.0004
GO:0043283	Biopolymer metabolic process	4.71	0.31	$5E^{-05}$	0.0006
P versus non-P[a]					
GO:0048589	Developmental growth (the increase in size or mass of an entire organism)	28.57	1.21	$4E^{-03}$	$8.E^{-02}$
GO:0045014	Negative regulation of transcription by Glc	28.57	1.21	$4E^{-03}$	$8.E^{-02}$
GO:0009733	Response to auxin stimulus	28.57	1.21	$4E^{-03}$	$8.E^{-02}$
GO:0002237	Response to molecule of bacterial origin	28.57	1.21	$4E^{-03}$	$8.E^{-02}$
GO:0010311	Lateral root formation	28.57	1.21	$4E^{-03}$	$8.E^{-02}$

[a]Non-AOP, all F-box genes in Arabidopsis, *Oryza*, and *Populus* excluding clade AOP; non-A, all F-box genes in Arabidopsis, *Oryza*, and *Populus* excluding clade A; non-P, all F-box genes in Arabidopsis, *Oryza*, and *Populus* excluding clade P. [b]Percentage of total query genes corresponding to a GO term indicates the proportion of GO (biological process)-annotated genes having that specific GO term in the clade AOP, A, or P. Percentage of total reference genes corresponding to a GO term indicates the proportion of GO (biological process)-annotated genes having that specific GO term in the non-AOP, non-A, or non-P F-box gene. [c]Unadjusted *P* values were obtained by means of a Fisher's exact test without adjusting for multiple comparisons to detect overrepresented GO terms in the clade AOP, A, or P, with non-AOP, non-A, or non-P, respectively, as a reference. Adjusted *P* values were the false discovery rate correction-adjusted *P* values obtained by Fisher's exact test.

growth signaling have expanded in *Populus* while those related to floral organ function have not. The modes of evolution of the gene families also varied among the examined species, where the F-box gene family appears to have predominantly expanded due to tandem duplication events in annual plants compared with the perennial *Populus*. Future studies employing proteomics and functional genomics approaches will be required to define the overall impact of gene family size, subgroup composition, and individual F-box genes on ubiquitination activity at the cellular level and the associated plant processes at the whole-organism level.

MATERIALS AND METHODS

Gene Identification and Annotation

A HMM profile multiple sequence alignment of 510 protein sequences for the F-box domain (PF00646) was downloaded from Pfam. HMMER (Eddy, 1998) was used to search a customized database containing the genome annotations of Arabidopsis (*Arabidopsis thaliana*; TAIR release 7; http://www.arabidopsis.org/), *Oryza* (*Oryza sativa*; TIGR release 5; http://rice.plantbiology.msu.edu/), *Populus* (*Populus trichocarpa*; JGI release 1.1; http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html), *Vitis* (*Vitis vinifera*; http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/; Jaillon et al., 2007), and *Carica* (*Carica papaya*; ftp://asgpb.mhpcc.hawaii.edu/papaya/annotation.genbank_submission/; Ming et al., 2008) for matches to the HMM profile with the threshold set at 1/100 of the Pfam GA gathering cutoff. The HMMER-selected proteins were then scanned for F-box domains using HMMPfam, HMMsmart, and ProfileScan implemented in InterPro (Mulder et al., 2007). The F-box-containing proteins identified by InterProScan were used for a BLASTp query (with an e-value cutoff of 1×10^{-20}) of the original protein database used for the HMMER search. Finally, the BLASTp hits were scanned for F-box domains using HMMPfam, HMMsmart, and ProfileScan implemented in InterPro (Mulder et al., 2007).

Our HMMER-BLASTp-InterProScan strategy initially identified 656, 699, and 336 F-box-containing genes in the genomes of Arabidopsis, *Oryza*, and *Populus*, respectively. Of the 699 *Oryza* F-box genes, 21 were transposable elements according to TIGR annotation (http://rice.plantbiology.msu.edu/), and they were excluded from the list of F-box proteins used for downstream analyses. Of the 336 *Populus* F-box genes, 17 genes were deleted because they appeared to represent gene duplicates found on small, unassembled scaffolds with no representation on the JGI *Populus* v1.1 VISTA browser (http://pipeline.lbl.gov/cgi-bin/gateway2?bg=ptr2filt&selector=vista) or because the gene

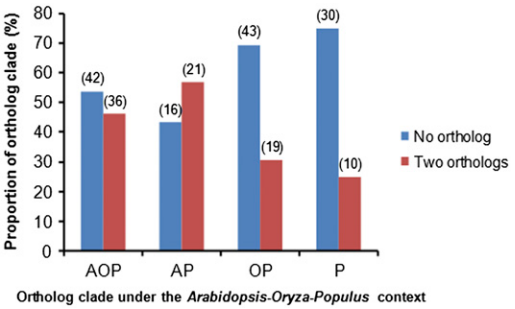

Figure 5. Comparison of F-box orthologs in *Vitis-Carica-Populus* within the Arabidopsis-*Oryza-Populus* context (note that groups with three or more *Populus* genes were excluded). The values in parentheses are numbers of *Populus* F-box genes. Under the Arabidopsis-*Oryza-Populus* context: AOP represents *Populus* F-box genes having orthologs in Arabidopsis and *Oryza*; AP represents *Populus* genes having orthologs in Arabidopsis only; OP represents *Populus* genes having orthologs in *Oryza* only; and P represents *Populus* genes having no orthologs in either Arabidopsis or *Oryza*. "No ortholog" represents *Populus* F-box genes having no orthologs in *Vitis* and *Carica*; "Two orthologs" represents two *Populus* genes co-orthologous to one or two genes in *Vitis* and/or *Carica*.

model sequences were truncated by captured gaps. The 319 *Populus* F-box genes (represented by a Jamboree gene model in the JGI official release) were checked manually using the JGI *Populus* genome browser (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html) to determine whether or not an alternative gene model better represented each gene. The final gene model was chosen based on the criteria of full length (with start and stop codons), longer transcript/coding region, and, most importantly, higher homology with Arabidopsis proteins. As such, 120 *Populus* Jamboree-predicted gene models were replaced by 121 better alternative gene models. (Note that the genomic region of a predicted gene model, fgenesh4_pm.C_LG_VI000041, overlapped two alternative F-box genes in tandem and was consequently replaced by those models, eugene3.00060123 and eugene3.00060124.) Therefore, the final *Populus* F-box gene list contains 320 genes (Supplemental Table S1).

For other substrate-specific E3 ligase gene families, such as HECT, RING, U-box, CDC20, and BTB, the Arabidopsis genes documented by Mazzucotelli et al. (2006) were used as queries to search a customized database containing the genome annotations of Arabidopsis (TAIR release 7; http://www.arabidopsis.org/), *Oryza* (TIGR release 5; http://rice.plantbiology.msu.edu/), and *Populus* (JGI release 1.1; http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html) by BLASTp with an e-value cutoff of 1×10^{-20}. The protein sequences of the BLASTp hits were scanned by InterPro (Mulder et al., 2007) for the signature protein domains: IPR000569 (HECT) for the HECT family, IPR001841 (zinc finger, RING type) or IPR013083 (zinc finger, RING/FYVE/PHD type) for the RING family, IPR003613 (U-box) for the U-box family, IPR000002 (Cdc20/Fizzy) for the CDC20 family, and IPR000210 (BTB/POZ-like), IPR013069 (BTB/POZ), or IPR011333 (BTB/POZ fold) for the BTB family.

Phylogenetic Tree Construction

Sequence alignments were performed with MAFFT (Katoh et al., 2005). The phylogenetic tree was constructed using the relaxed neighbor-joining method (Evans et al., 2006). Bootstrap analysis was performed with SEQBOOT and CONSENSUS in the PHYLIP package (Felsenstein, 1989). The gene tree was reconciled with a species tree ([[Arabidopsis, *Populus*], *Oryza*] or [[*Carica*, *Populus*], *Vitis*]) using Notung (Chen et al., 2000) to estimate upper and lower bounds of the time of duplication. The tree was displayed using MEGA version 4.0 (Tamura et al., 2007). Orthologs, the genes originating from a single ancestral gene in the last common ancestor of the compared genomes (Koonin, 2005), were identified according to the reconciled phylogenetic trees.

Localization of F-Box Genes in the Genome

F-box gene distribution among chromosomes was evaluated by the observed number of F-box genes compared with their expected number under a Poisson distribution. The expected gene number λ_i on chromosome i would be a sample from a Poisson distribution, $\lambda_i = mL_i/\sum L_i$, where, m is the total number of genes detected within the assembled sequences and L_i is the length of chromosome i. The probabilities $p(m_i < \lambda_i)$ and $p(m_i > \lambda_i)$ were evaluated under the cumulative Poisson distribution at $\alpha \leq 0.05$ and $\alpha \leq 0.01$ significance levels.

Identification of Duplicated Genes

The identification of homologous chromosome segments in *Populus* resulting from whole-genome duplication events was described by Tuskan et al. (2006). Blocks of the same color represent the homologous chromosome segments. The information for Arabidopsis gene duplication was obtained from ftp://ftp.tigr.org/pub/data/a_thaliana/ath1/DATA_RELEASE_SUPPLEMENT/. The information for *Oryza* segmental duplication was obtained from http://rice.plantbiology.msu.edu/segmental_dup/100kb/segdup_100kb.shtml. The tandemly duplicated genes in *Oryza* were identified and defined as an array of two or more genes with Smith-Waterman alignment e-values $\leq 1 \times 10^{-25}$ that were enclosed within a 100-kb window. The analysis of *Populus* tandem gene duplication, obtained from Tuskan et al. (2006), used the same criteria as for *Oryza* with added inclusion of maximum 4FTV = 1. Segmental duplications in *Populus* were identified by BLASTp as described for *Oryza*, but the expectation value was raised to $e = 1e^{-25}$. Protein alignments of fewer than 50 amino acids were excluded. Segmentally duplicated pairs of *Populus* genes identified by BLASTp were verified as true paralogs using the VISTA browser (http://pipeline.lbl.gov/cgi-bin/gateway2?bg=ptr2filt&selector=vista) *Populus* duplicates track with default settings (minimum conserved region width = 100 bp; conservation identity = 70%) to confirm homology.

Homology Search in Other Plant Species

The 1,654 F-box protein sequences identified in Arabidopsis, *Populus*, and *Oryza* were used to query against transcript assemblies from 193 plant species (Childs et al., 2007) using tBLASTn with e-value cutoffs of $1e^{-10}$, $1e^{-20}$, $1e^{-30}$, $1e^{-40}$, $1e^{-50}$, $1e^{-60}$, $1e^{-70}$, and $1e^{-80}$ (Supplemental Table S7). Differences in the distribution of F-box genes among the ortholog clades (i.e. AOP, AO, OP, AP, A, O, and P) between the initial query F-box genes and the queries that have BLAST hits decreased with an increase of stringency in the e-value cutoff (from $1e^{-10}$ to $1e^{-80}$). This pattern in ortholog clade distribution was caused by a faster decrease in the percentage of the ortholog clades of F-box proteins in the species-specific clades (A, O, and P), suggesting that the phylogenetic signal was decaying more quickly in these clades (Supplemental Fig. S4). The ortholog clade distribution of the F-box proteins having BLAST hits became significantly ($P \leq 1e^{-7}$) different from the ortholog clade distribution of all of the query F-box proteins at an e-value cutoff of $1e^{-30}$. This e-value cutoff was used to investigate the distribution of BLAST hits (the F-box gene homologs) among herbaceous monocot, herbaceous eudicot, and woody eudicot samples derived from the transcript assemblies of more than 250 plant species (Childs et al., 2007).

Identification of Protein Motifs

Protein sequences were scanned for domains using BlastProDom, FPrintScan, HMMPIR, HMMPfam, HMMsmart, HMMTigr, ProfileScan, ScanRegExp, and SuperFamily implemented in InterPro (Mulder et al., 2007).

Intron Analysis

Intron information was obtained from the TAIR Arabidopsis annotation release 7 (http://www.arabidopsis.org/), TIGR *Oryza* annotation release 5 (http://rice.plantbiology.msu.edu/), and JGI *Populus* annotation release 1.1 (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html).

Expression Evidence of F-Box Genes

Expression evidence from ESTs or full-length cDNAs for Arabidopsis genes was obtained from TAIR release 7 (http://www.arabidopsis.org/). Expression evidence from ESTs or full-length cDNAs for *Oryza* genes was obtained from TIGR release 5 (http://rice.plantbiology.msu.edu/). Expression evidence from ESTs or full-length cDNAs for *Populus* genes was determined by a minimum of 97% identity over an alignment of at least 100 bp and at least 80% length of the shorter sequences.

Analysis of Gene Expression

Two Arabidopsis microarray data sets were compiled from AtGenExpress (Schmid et al., 2005; Kilian et al., 2007). The developmental data set is represented by the following organs/tissues: cotyledons, hypocotyls, roots, shoot apices, rosette leaves, senescing leaves, second internodes, flowers, sepals, petals, stamens, carpels, siliques, and seeds. The gene expression levels are expressed as $LOG_2(x/y)$, where x is the detection signal from the above tissue types and y is the detection signal from seedlings. The environmental data set is represented by the following treatments: cold, salt, drought, oxidative, UV-B light, heat, pathogen stresses, and blue light, far-red light, red light, and white light environments. Dark treatment was used as a control for the light experiments. See Kilian et al. (2007) and Schmid et al. (2005) for further details. K-means clustering of the Arabidopsis microarray data was performed using EPCLUST (http://ep.ebi.ac.uk/EP/EPCLUST/) with correlation distance (uncentered).

Coexpression of F-Box-Related Genes

In addition to the F-box genes, there are also several other F-box-related genes involved in the SCF complex, including CAND1, COP9, Cul1, E1, E2, RBX1, ROC1, RUB1/2, and SKP1/ASK1/ASK2 (Lechner et al., 2006). In order to compare F-box-related gene expression across 12 *Populus* tissues with the expression of the 320 F-box genes (Gene Expression Omnibus Database under accession nos GSM146141 to GSM146299; series GSE6422; platform GPL2618), a Spearman's rank correlation was performed. F-box-related genes in Arabidopsis were first identified by querying the gene names in the TAIR database (Rhee et al., 2003), and subsequent sequence information was used to perform a BLASTp analysis on the JGI *Populus* v1.1 browser (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html). This resulted in a list of 146 *Populus* F-box-related genes, the names of which were used to query NimbleGen whole-genome microarray data from 12 different *Populus* tissues. Those genes expressed significantly above background ($Q \leq 0.05$) were said to be expressed in a particular tissue. Microarray data from the 320 F-box genes and F-box-related genes were then compared to see if the number of genes expressed in each tissue occurred in a similar pattern across the 12 tissues. A Spearman's rank correlation was calculated.

GO Analysis

GO annotation of the F-box proteins was performed using BLAST2GO, with parameters optimized for the annotation of Arabidopsis sequences (National Center for Biotechnology Information nonredundant database; 20 hits maximum and 33 amino acid minimum high scoring pair length; e-value hit filter of $1e^{-06}$; annotation cutoff value of 55; GO weight of 5; Conesa et al., 2005). GO enrichment analysis was performed using FatiGO+ (Al-Shahrour et al., 2007).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Phylogenetic tree of the F-box genes in Arabidopsis, *Oryza*, and *Populus*.

Supplemental Figure S2. Phylogenetic tree of the F-box genes in *Carica*, *Populus*, and *Vitis*.

Supplemental Figure S3. Ortholog clades of well-characterized Arabidopsis genes.

Supplemental Figure S4. The percentage of the ortholog clades of F-box proteins in Arabidopsis, *Oryza*, and *Populus* showing homology to other plant species.

Supplemental Table S1. F-box genes in Arabidopsis, *Oryza*, and *Populus*.

Supplemental Table S2. Well-characterized Arabidopsis F-box genes.

Supplemental Table S3. The herbaceous monocot, herbaceous eudicot, and woody eudicot species.

Supplemental Table S4. Domain structure of the F-box proteins in Arabidopsis, *Populus*, and *Oryza*.

Supplemental Table S5. F-box genes in *Carica* and *Vitis*.

Supplemental Table S6. Arabidopsis S-locus F-box genes by phylogenetic group and orthologous clade.

Supplemental Table S7. Number of F-box proteins in Arabidopsis, *Oryza*, and *Populus* showing homology to other plant species.

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