The Repertoire of Transfer RNA Genes Is Tuned to Codon Usage Bias in the Genomes of *Phytophthora sojae* and *Phytophthora ramorum*

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Submitted 11 April 2006. Accepted 7 August 2006.

In all, 238 and 155 transfer (t)RNA genes were predicted from the genomes of *Phytophthora sojae* and *P. ramorum*, respectively. After omitting pseudogenes and undetermined types of tRNA genes, there remained 208 *P. sojae* tRNA genes and 140 *P. ramorum* tRNA genes. There were 45 types of tRNA genes, with distinct anticodons, in each species. Fourteen common anticodon types of tRNAs are missing altogether from the genome in the two species; however, these appear to be compensated by wobbling of other tRNA anticodons in a manner which is tied to the codon bias in *Phytophthora* genes. The most abundant tRNA class was arginine in both *P. sojae* and *P. ramorum*. A codon usage table was generated for these two organisms from a total of 9,803,525 codons in *P. sojae* and 7,496,598 codons in *P. ramorum*. The most abundant codon type detected from the codon usage tables was GAG (encoding glutamic acid), whereas the most numerous tRNA gene had a methionine anticodon (CAT). The correlation between the frequencies of tRNA genes and the codon frequencies in protein-coding genes was very low (0.12 in *P. sojae* and 0.19 in *P. ramorum*); however, the correlation between amino acid tRNA gene frequency and the corresponding amino acid codon frequency in *P. sojae* and *P. ramorum* was substantially higher (0.53 in *P. sojae* and 0.77 in *P. ramorum*). The codon usage frequencies of *P. sojae* and *P. ramorum* were very strongly correlated (0.99), as were tRNA gene frequencies (0.77). Approximately 60% of orthologous tRNA gene pairs in *P. sojae* and *P. ramorum* are located in regions that have conserved synteny in the two species.

Additional keywords: tRNA gene copy number.

Codon usage bias, defined as the unequal usage of synonymous codons for encoding amino acids, first was described by Grantham and colleagues (Grantham et al. 1981). The possibility of relationships among synonymous codon usage, transfer (t)RNA availability, and the rate of protein synthesis was studied even earlier (Zuckerandl and Pauling 1965).

Codon usage bias has been found in many organisms, both prokaryotic and eukaryotic. This bias varies considerably among organisms and even within genes of the same organism. In several different species, codon usage bias was found to be

correlated weakly with gene expression level. Previous codon usage analyses showed associations with various biological factors, such as gene expression level (Bulmer 1987; Eyre-Walker 1996), gene length (Wan et al. 2003), gene translation initiation signal (Ma et al. 2002), protein amino acid composition (D'Onofrio et al. 1991; Lobry and Gautier 1994), and tRNA abundance (Bulmer 1987; Ikemura 1982; Kanaya et al. 1999). A major driving force responsible for deviations from a neutral choice of codons is translational selection, which results in adjustment of codon usage to the abundance of the various tRNA species. Whole-genome analysis in the yeast *Saccharomyces cerevisiae* revealed that codon usage and protein amino acid composition were adapted to both gene copy number and intracellular content of individual tRNA species (Percudani et al. 1997). Although the biased usage of synonymous codons is, to a large extent, explained by this mutual relationship, the preference for one codon over another also may depend on more intrinsic aspects of the translational machinery. One such aspect is the stability of the codon–anticodon interaction. The contribution of codon–anticodon complex stability to translational selection can be evaluated by measuring the relative usage of cognate codons (i.e., codons read by a single tRNA species), whose choice is independent of tRNA abundance (Percudani and Ottonello 1999).

Most of the work on codon usage bias has been focused on the analysis of codon frequencies from the DNA sequences of protein-coding genes. A complementary approach is to examine the distribution of tRNA gene number and anticodon type. With the completion of the draft genome sequences of the oomycete pathogens *Phytophthora sojae* and *P. ramorum*, we have had the opportunity to study the complete tRNA gene sets from these two pathogens and to assess the relationship of tRNA gene distribution with codon usage. In this study, we present complete codon usage tables for these two organisms along with evidence, based on tRNA gene copy numbers, suggesting that selection acts on codon usage bias and on the tRNA gene repertoire in the *Phytophthora* genomes.

RESULTS AND DISCUSSION

We performed a genomewide analysis of tRNA gene distribution for *P. sojae* and *P. ramorum* to understand codon bias as well as the relative frequencies of tRNA genes in these organisms. In all, 238 tRNA genes were predicted in *P. sojae* and 155 tRNA genes were predicted in *P. ramorum*. Of the 238 predicted genes in *P. sojae*, 16 were pseudo-tRNA genes (without any detectable secondary structure) and 14 were predicted tRNA genes of undetermined type. In *P. ramorum*, of the 155 predicted genes, 11 were pseudogenes and 4 were of undetermined type.

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Isoaccepting tRNA types.

The various types of tRNAs carrying a specific type of amino acid are called isoaccepting tRNAs. Gene copy numbers vary greatly among isoaccepting tRNAs. For both *P. sojae* and *P. ramorum*, all 20 isoaccepting tRNA classes were found, representing 47 tRNA genes. Cysteine tRNA genes were missing from the *P. sojae* sequence assembly and tryptophan tRNA genes were missing from the *P. ramorum* assembly. However, when the trace archives were searched, three cysteine genes were found in *P. sojae*, and one tryptophan tRNA gene in *P. ramorum*. The highest tRNA gene copy number found in both the species was for arginine, whereas the most numerous single-codon-type tRNA gene was methionine.

Codon preference.

Codon usage for *P. sojae* and *P. ramorum* is illustrated in Table 1. The correlation between the codon usage frequencies of *P. sojae* and *P. ramorum* was highly significant ($r = 0.99$) (Fig. 1).

Phytophthora spp. show strong codon usage bias. As has been noted previously (Jiang and Govers 2006; Randall et al. 2005), codon usage in *Phytophthora* spp. is biased heavily toward use of G and C in the third position, with the exception that GGG is used relatively rarely. Also, T is preferred over A in the third position. The most frequent codon for both organisms is GAG coding for glutamic acid. Unlike many other higher organisms where Asp codons do not exhibit codon usage bias (Moriyama and Powel 1997), *Phytophthora* Asp codons are strongly biased toward GAC over GAT. The least frequent codon in the two species is ATA, which codes for isoleucine.

In addition to the third base bias toward G or C, the presence of G, C, or both in the first two positions also is slightly more favored in both *P. sojae* and *P. ramorum* (27 and 26%, respectively, of total codon frequency) over the presence of A, T, or both in the first two places (20 and 22%, respectively, of total codon frequency). This observation is similar to earlier observation that GC-rich genomes tend to favor GC-rich codons over AT-rich codons (Knight et al. 2001).

Absence of specific anticodon tRNA types is tuned to the codon usage bias.

There are a total of 208 confirmed tRNA genes in *P. sojae* and 140 tRNA genes in *P. ramorum*. The confirmed tRNA genes present in each organism represent 45 different tRNA gene anticodons. However, 16 anticodons were missing from the *P. sojae* and *P. ramorum* tRNA gene repertoires, of which 14 anticodons were missing from both (Table 2, in bold). Seven of the missing anticodon types (GGC, GCG, GAG, GGG, GGA, CGU, and GAC) (Table 2, square brackets) correspond to codons with a high relative frequency of usage (0.034, 0.026, 0.023, 0.013, 0.013, 0.026, and 0.02 in *P. sojae* and 0.021, 0.018, 0.017, 0.008, 0.01, 0.02, and 0.014 in *P. ramorum*).

The explanation for the missing tRNA classes appears to be related to functional redundancy among tRNA genes. Many tRNAs can pair with more than one codon as a result of wobble pairing (usually, G-U pairing) and modifications of anticodon bases (usually, deamination of A to form inosine, which can pair with all four bases). In the presence of functional redundancy, nonessential tRNA genes or their corresponding amino-acyl tRNA synthetases may be lost due to mutation. In the case of the two *Phytophthora* spp., the loss of the tRNA genes appears to be constrained by the codon usage bias toward C and G in the third position.

In every case but one in which an amino acid is encoded by a codon with C or U in the third position, including the serine

codons AGC and AGU, the tRNA class corresponding to U in the third position is missing. Therefore, for these amino acids, only tRNAs with G in the first position of the anticodon are available to decode both NNC and NNU codons. Because the G-C pairing is more stable than the G-U wobble pairing, these tRNAs presumably bind more efficiently to the NNC codons. There is a strong bias toward use of NNC codons over NNU codons; therefore, the loss of the ANN tRNA genes presumably has conferred no selective disadvantage. The only exception is phenylalanine tRNAs in which both GAA and AAA anticodons are present.

In the case of amino acids in which there are three or four possible bases in the third position, including serine UCN codons, leucine CUN codons, and arginine CGN codons, in all but one instance (glycine), tRNAs with GNN anticodons are missing. Presumably, therefore, the NNC specificity is provided, with no selective disadvantage, by tRNAs with ANN anticodons that are converted to INN. Inosine has the ability to pair with all four of the conventional bases; however, the stability of pairing is substantially higher for the pairing with C than with any of the other bases and poorest for the pairing with G (Watkins and SantaLucia 2005). Therefore, tRNAs with INN anticodons will efficiently translate NNC codons, resulting in the eventual loss of tRNAs with GNN anticodons. On the other hand, the poor pairing of INN with NNG codons combined with the high abundance of NNG codons presumably has prevented the loss of CNN tRNAs dedicated to decoding NNG codons. The efficiency of pairing of INN anticodons with NNA codons also appears to be sufficiently poor that tRNA genes with UNN anticodons have been retained, despite the fact that NNA codons are the least frequent.

For amino acids encoded by codons with purines in the third position, including leucine UUR codons and arginine AGR codons, there is no case in which a codon specificity is missing. G can base pair with U; therefore, in principle, tRNAs with UNN anticodons could decode both NNG and NNA codons. However, in that case, the more stable pairing of A to U over G to U would favor pairing with the least common base found at the third position in *Phytophthora* codons, A, over pairing with the more common G. This presumably has created selection for the retention of tRNA genes with CNN anticodons. Therefore, the consistent presence of tRNAs with CNN anticodons specialized for decoding NNG codons supports the hypothesis that the presence and absence of tRNA specificities in the *Phytophthora* genomes is tuned to the strong bias toward G and C in the third codon position, as a result of selection pressure to retain only those tRNA genes that are essential for efficiently translating the most common codons.

Together, these observations reveal that, in these two *Phytophthora* spp., extensive use of adenosine to inosine conversion in the 5' end of the tRNA anticodon and wobble pairing has resulted in a streamlined repertoire of tRNA genes tightly tuned to the third codon position preference for G and C.

Correlation between codon frequency and tRNA gene copy number.

In both species, the most abundant class of tRNA gene, by amino acid specificity, was the arginine-tRNA genes. There are 30 arginine tRNA genes in *P. sojae* and 17 in *P. ramorum*. The next most abundant tRNA genes in both species were leucine tRNA genes. The most abundant tRNA gene in both species, by anticodon sequence, was the methionine tRNA gene. In contrast, the most frequently used single codon in the genomes was GAG coding for glutamic acid (46.685 of 1,000 in *P. sojae* and 46.103 of 1,000 in *P. ramorum*). There was a positive correlation between the number of tRNA genes for each amino

Table 1. Codon usage table in *Phytophthora sojae* and *P. ramorum* calculated after predicting 9,803,525 codons in *P. sojae* and 7,496,598 codons in *P. ramorum* from the coding region

Codon	Amino acid	<i>P. sojae</i>				<i>P. ramorum</i>			
		Freq. ^a	Number per 1,000			Freq. ^a	Number per 1,000		
			Codons ^b	Amino acids ^c	Total ^d		Codons ^b	Amino acids ^c	Total ^d
GCT	Ala	0.212	20.915	98.79	204,274	0.221	20.796	93.877	155,555
GCG	Ala	0.338	33.357	...	325,793	0.316	29.775	...	222,713
GCA	Ala	0.147	14.501	...	141,628	0.17	16.008	...	119,741
GCC	Ala	0.304	30.017	...	293,168	0.292	27.516	...	205,816
CGT	Arg	0.152	10.985	72.324	107,296	0.177	11.056	62.249	82,700
CGG	Arg	0.162	11.683	...	114,111	0.147	9.147	...	68,423
AGG	Arg	0.092	6.642	...	64,874	0.072	4.467	...	33,415
CGA	Arg	0.158	11.437	...	111,706	0.16	9.989	...	74,719
AGA	Arg	0.073	5.273	...	51,509	0.066	4.087	...	30,573
CGC	Arg	0.364	26.304	...	256,906	0.379	23.645	...	176,866
AAC	Asn	0.78	24.225	31.039	236,597	0.749	24.858	33.113	185,934
AAT	Asn	0.22	6.814	...	66,554	0.251	8.332	...	62,326
GAC	Asp	0.733	41.224	56.236	402,626	0.701	40.595	57.764	303,647
GAT	Asp	0.267	15.012	...	146,618	0.299	17.303	...	129,424
TGT	Cys	0.27	4.691	17.348	45,822	0.276	4.399	15.913	32,905
TGC	Cys	0.73	12.657	...	123,623	0.724	11.551	...	86,402
CAG	Gln	0.708	29.161	41.189	284,809	0.706	29.647	41.882	221,757
CAA	Gln	0.292	12.028	...	117,481	0.294	12.332	...	92,244
GAG	Glu	0.724	46.685	64.487	455,963	0.694	46.103	66.265	344,849
GAA	Glu	0.276	17.802	...	173,870	0.306	20.314	...	151,951
GGG	Gly	0.165	10.576	63.927	103,292	0.148	9.431	63.615	70,548
GGC	Gly	0.459	29.37	...	286,848	0.445	28.389	...	212,350
GGA	Gly	0.201	12.833	...	125,339	0.201	12.786	...	95,640
GGT	Gly	0.174	11.148	...	108,881	0.206	13.154	...	98,392
CAC	His	0.746	17.615	23.6	172,044	0.749	17.281	23.01	129,261
CAT	His	0.254	5.985	...	58,456	0.251	5.782	...	43,254
ATT	Ile	0.261	9.264	35.451	90,481	0.311	11.868	38.051	88,775
ATA	Ile	0.054	1.9	...	18,589	0.047	1.811	...	13,550
ATC	Ile	0.685	24.287	...	237,203	0.641	24.46	...	182,958
CTT	Leu	0.1	8.927	89.104	87,192	0.11	10.211	92.294	76,378
TTG	Leu	0.148	13.149	...	128,428	0.165	15.292	...	114,384
TTA	Leu	0.025	2.194	...	21,429	0.026	2.425	...	18,139
CTA	Leu	0.058	5.166	...	50,464	0.061	5.599	...	41,882
CTC	Leu	0.261	23.248	...	227,055	0.245	22.649	...	169,413
CTG	Leu	0.409	36.42	...	355,707	0.393	36.332	...	271,762
AAG	Lys	0.838	40.265	48.046	393,254	0.794	39.727	49.922	297,153
AAA	Lys	0.162	7.781	...	75,998	0.206	10.311	...	77,126
ATG	Met	1	22.847	22.847	223,139	1	24.573	24.516	183,805
TTC	Phe	0.787	26.895	34.182	262,682	0.732	27.224	37.108	203,635
TTT	Phe	0.213	7.287	...	71,173	0.268	9.97	...	74,574
CCT	Pro	0.177	9.294	52.382	90,779	0.188	8.977	47.523	67,150
CCG	Pro	0.398	20.829	...	203,433	0.391	18.618	...	139,262
CCA	Pro	0.174	9.104	...	88,919	0.184	8.766	...	65,572
CCC	Pro	0.251	13.155	...	128,487	0.237	11.27	...	84,303
TCT	Ser	0.094	7.746	82.276	75,661	0.102	8.277	80.826	61,917
TCG	Ser	0.292	24.027	...	234,665	0.287	23.251	...	173,915
AGC	Ser	0.258	21.207	...	207,126	0.245	19.818	...	148,242
TCA	Ser	0.085	6.952	...	67,904	0.088	7.134	...	53,362
TCC	Ser	0.166	13.69	...	133,714	0.161	13.045	...	97,577
AGT	Ser	0.105	8.654	...	84,527	0.117	9.48	...	70,951
ACT	Thr	0.16	9.368	58.727	91,496	0.158	9.404	59.224	70,345
ACC	Thr	0.261	15.334	...	149,770	0.246	14.579	...	109,053
ACA	Thr	0.125	7.353	...	71,819	0.145	8.601	...	64,335
ACG	Thr	0.454	26.672	...	260,505	0.451	26.776	...	200,284
TAC	Tyr	0.81	20.064	24.765	195,959	0.794	20.647	25.934	154,439
TAT	Tyr	0.19	4.701	...	45,915	0.206	5.347	...	39,999
TGG	Trp	1	13.458	13.458	131,446	1	12.445	12.416	93,087
GTT	Val	0.132	9.184	69.791	89,697	0.139	10.084	72.172	75,432
GTG	Val	0.504	35.158	...	343,376	0.523	37.837	...	283,018
GTA	Val	0.068	4.722	...	46,121	0.074	5.375	...	40,208
GTC	Val	0.297	20.727	...	202,437	0.263	19.042	...	142,431
TAA	Stop	0.225	...	...	8,282	0.297	...	...	4,983
TAG	Stop	0.241	...	...	8,907	0.313	...	...	5,244
TGA	Stop	0.534	...	...	19,698	0.39	...	...	6,550

^a Relative synonymous codon usage frequency.

^b Number of codons per 1,000 codons.

^c Sum of codons of a particular amino acid per 1,000 codons.

^d Total number of codons for the whole genome.

acid in *P. sojae* and *P. ramorum* ($r = 0.76$). The copy number typically was higher in *P. sojae* than in *P. ramorum*, except for a few cases where there was a significantly higher number of tRNA genes present for *P. ramorum* than *P. sojae* (Fig. 2). The alanine, phenylalanine, and lysine tRNA genes were more numerous in *P. ramorum* than in *P. sojae*. There existed a very weak correlation between the codon usage frequencies in the protein-coding regions and the corresponding tRNA gene frequencies ($r = 0.12$ in *P. sojae* and $r = 0.19$ in *P. ramorum*). This is in contrast to the earlier established hypothesis that there is strong correlation between the two (Percudani et al.

Fig. 1. Amino acid codon usage frequency of *Phytophthora sojae* versus *P. ramorum* (■), $r = 0.99$, and amino acid transfer (t)RNA gene frequency of *P. sojae* versus *P. ramorum* (◇), $r = 0.7$.

1997). Even considering the codon usage frequencies of highly expressed protein-coding genes, the correlation coefficient was even lower ($r = 0.09$) in *P. sojae*. This lack of correlation is expected if there is extensive use of anticodon modification and wobble pairing in matching tRNAs to codons during translation. Consistent with this explanation, the correlation improved significantly if calculated after pooling the tRNA gene numbers for each amino acid type (amino acid tRNA gene frequencies) and pooling the codon usage frequencies for each amino acid (amino acid codon frequencies) (Fig. 3) ($r = 0.53$ and 0.77 in *P. sojae* and *P. ramorum*, respectively). The differences in the correlations between the two species could be due to differences in the transcription rates of the relevant tRNA genes or the loading efficiencies of the relevant amino acyl-tRNA synthetases. In many unicellular (Kanaya et al. 1999; Percudani et al. 1997) as well as multicellular organisms (Duret 2000), tRNA gene copy number serves as a direct indicator of tRNA content in the cell and correlates strongly with the codon usage ($r = 0.82$ in *Caenorhabditis elegans* and $r = 0.82$ in yeast). However, studies are lacking on genomes high in GC content to conclusively prove whether wobbling is more frequent in GC rich genomes.

Spatial organization and synteny between the tRNA coding genes.

The tRNA genes were organized in loose clusters of two or three throughout the genome. In a few cases (Gln, Glu, Gly, and Leu in *P. sojae* and Met, Lys, Glu, and Ala in *P. ramorum*), they occurred as clusters of more than four. Very little is known about the genomic organization and chromosomal location of tRNA genes in *Phytophthora* spp. In some eukaryotes, tRNA

Fig. 2. Transfer (t)RNA gene frequency (upright) and codon usage frequency (inverted) in *Phytophthora sojae* (■) and *P. ramorum* (□).

genes are dispersed as single copies (Arnold et al. 1986), whereas others are clustered (Müller et al. 1987; Shortridge et al. 1989). The significance of tRNA gene organization and expression needs to be investigated further.

There has been extensive divergence between the *P. sojae* and *P. ramorum* genomes. Nevertheless, most of the orthologous protein coding genes in the *P. sojae* and *P. ramorum* genomes have syntenic locations (Tyler et al. 2006). By comparison, approximately 59 of the 104 pairs of orthologous tRNA genes have syntenic locations, based on the presence of syntenic orthologous protein-coding genes nearby. In several cases, tRNA gene families in syntenic locations have more members in one species than another. Therefore, 68 and 71 tRNA genes in *P. sojae* and *P. ramorum*, respectively, have locations syntenic with the other species. The reduced synteny of the tRNA genes suggests that whatever evolutionary forces have constrained the protein-coding genes to syntenic locations in the face of extensive genomic diversification have not constrained the tRNA genes so strongly.

Conclusion.

We have confirmed, at a genomewide level, the strong bias toward G and C in the third codon position in *Phytophthora* spp., and we also have identified a bias toward codons with G and C in the first and second positions. We have cataloged all the tRNA genes in *P. sojae* and *P. ramorum*. A consistent absence of tRNA genes with specific anticodons (GNN for amino

acids with fourfold redundancy and ANN for amino acids with twofold redundancy) revealed that the two *Phytophthora* spp. utilize streamlined sets of tRNA genes tuned to the preference for G and C in the third codon position. In concert with this observation, a weak correlation between individual codon frequency and isoaccepting tRNA gene frequency indicates that wobble-pairing is used very extensively.

Different tRNA genes often occur in spatial clusters in the two species, suggesting a link between the organization of tRNA genes with their transcription level. On the other hand, the percentage of orthologous tRNA genes located in syntenic regions of the two genomes was less than that for orthologous protein-coding genes (60 compared with 90%), suggesting a reduced importance of genome position compared with protein-coding genes.

MATERIALS AND METHODS

The genome sequences of *P. sojae* and *P. ramorum* (Tyler et al. 2006) were obtained through a collaboration with the Department of Energy Joint Genome Institute. For *P. sojae*, a total of 9,803,525 codons was taken from the entire transcript set (approximately 19,000) and unigenes derived from the expressed sequence tags (ESTs). In the case of *P. ramorum*, the entire transcript dataset (15,743 transcripts and 7,496,598 codons) was used. To calculate the codon usage data, coding sequences were passed through the CUSP program available in the EMBOSS package (Rice et al. 2000).

Table 2. Transfer (t)RNA gene distribution and codon degeneracy in *Phytophthora sojae* (Ps) and *P. ramorum* (Pr)

Amino acid	Species ^c	No. of functional tRNA genes ^a				Anticodon ^b		Codon specificity ^d	Relative tRNA gene copy no.		Codon usage	
		Ps	Pr			tDNA	tRNA		Ps	Pr	Ps	Pr
Ala	Ala1	4	10	3	14	AGC	IGC	GCU:[GCC]:GCA	0.019	0.021	0.021	0.021
	Ala2	3	...	5	...	CGC	CGC	CGG	0.014	0.036	0.033	0.029
	Ala3	3	...	6	...	TGC	UGC	GCA:GCG	0.014	0.043	0.014	0.016
	Ala4	0	...	0	...	GGC	GGC	GCC*	0	0	0.034	0.027
Arg	Arg1	2	30	3	17	ACG	ICG	CGU:[CGC]:CGA	0.009	0.021	0.011	0.011
	Arg2	7	...	4	...	CCG	CCG	CGG	0.033	0.028	0.011	0.009
	Arg3	5	...	3	...	CCT	CCU	AGG	0.024	0.021	0.006	0.004
	Arg4	13	...	5	...	TCG	UCG	CGA:CGG	0.062	0.036	0.011	0.009
	Arg5	3	...	2	...	TCT	UCU	AGA:AGG	0.014	0.014	0.005	0.004
	Arg6	0	...	0	...	GCG	GCG	CGC*	0	0	0.026	0.023
Asn	Asn1	2	2	1	1	GTT	GUU	AAC:[AAU]	0.009	0.007	0.024	0.024
	Asn2	0	...	0	...	ATT	AUU	AAU*	0	0	0.007	0.008
Asp	Asp1	5	5	3	3	GTC	GUC	GAC:[GAU]	0.024	0.021	0.041	0.040
	Asp2	0	...	0	...	ATC	AUC	GAU*	0	0	0.015	0.017
Cys	Cys1	0	3**	0	2	ACA	ACA	UGU*	0	0	0.004	0.004
	Cys2	3**	...	2	...	GCA	GCA	UGC:[UGU]	0.014	0.014	0.012	0.011
Gln	Gln1	10	20	4	7	CTG	CUG	CAG	0.048	0.028	0.029	0.029
	Gln2	10	...	3	...	TTG	UUG	CAA:CAG	0.048	0.021	0.012	0.012
Glu	Glu1	5	12	4	11	CTC	CUC	GAG	0.024	0.028	0.047	0.046
	Glu2	7	...	7	...	TTC	UUC	GAA:GAG	0.033	0.050	0.017	0.020
Gly	Gly1	3	13	1	11	CCC	CCC	GGG	0.014	0.007	0.010	0.009
	Gly2	6	...	4	...	GCC	GCC	GGC:GGU	0.029	0.028	0.029	0.028
	Gly3	4	...	4	...	TCC	UCC	GGA:GGG	0.019	0.028	0.012	0.012
	Gly4	0	...	2	...	ACC	ICC	GGU:GGC:GGA	0	0.014	0.011	0.013
His	His1	3	3	2	2	GTG	GUG	CAC:[CAU]	0.014	0.014	0.017	0.017
	His2	0	...	0	...	ATG	AUG	CAU*	0	0	0.006	0.006
Ile	Ile1	3	12	1	4	AAT	IAU	AUU:AUA: [AUC]	0.014	0.007	0.009	0.011
	Ile2	9	...	3	...	TAT	UAU	AUA	0.043	0.021	0.002	0.002
	Ile3	0	...	0	...	GAT	GAU	AUC*	0	0	0.024	0.024
Leu	Leu1	3	23	4	13	AAG	IAG	CUU:[CUC]:CUA	0.014	0.028	0.009	0.010
	Leu2	4	...	3	...	CAA	CAA	UUG	0.019	0.021	0.013	0.015
	Leu3	6	...	1	...	CAG	CAG	CUG	0.029	0.007	0.036	0.036

(continued on next page)

^a tRNA marked with ** are retrieved from the trace archives.

^b Anticodons marked in bold are not present in the organism.

^c tRNA species marked in bold are not present in the organism.

^d Codons in italics (but not in bold) may exhibit wobbling. Anticodons and codons in brackets represent possible wobbling after the 5' A of tDNA is converted to I in tRNA. Codons marked with an asterisk (*) are potentially provided by other isoaccepting tRNAs by means of wobbling.

The codon usage frequency was calculated by dividing the number of codons by the total number of codon minus stop codons. To establish a relationship between the codon frequency and tRNA gene copy number for highly expressed genes, 40 highly expressed genes were identified using *P. sojae* EST libraries.

The entire genome sequences of *P. sojae* (78,050,814 bases, from the 9X assembly) and *P. ramorum* (54,424,536 bases, from the 7X assembly) were passed through the tRNAscan-SE program (Lowe and Eddy 1997). tRNAscan-SE combines the specificity of the Cove probabilistic RNA prediction package (Eddy and Durbin 1994) with the speed and sensitivity of tRNAscan 1.3 (Fichant and Burks 1991). There were some tRNA types missing from the assemblies: cys-tRNA in *P. sojae* and trp-tRNA in *P. ramorum*. To check if the missing genes could be found among the unassembled reads, the tRNA sequences from the other species were searched with BLAST against the trace archives. In both cases, reads corresponding to the missing tRNA genes were found and were reassembled manually using DNA Strider. The whole gene list then was updated.

We have used the term “codon usage frequency” to denote the frequency of individual codons as a fraction of the total number of codons predicted in each genome. We have used the term “tRNA gene copy number” to denote the actual number of tRNA genes with an individual anticodon predicted by tRNAscan-SE in each genome and the term “tRNA gene frequency” to indicate

the number of tRNA genes with an individual anticodon divided by the total number of tRNA genes in each genome (we have not included the pseudo and nondetermined tRNA types for this). For some analyses, the codon frequencies and tRNA gene frequencies were pooled for each amino acid. In these cases, we have used the terms “amino acid codon frequencies” and “amino acid tRNA gene frequencies.”

Correlation coefficients of different groups were calculated and plotted in Microsoft Excel. All the programs were run on a

Fig. 3. Amino acid transfer (t)RNA gene frequency versus amino acid codon frequency in *Phytrophthora sojae* (■), $r = 0.533$, and *P. ramorum* (◇), $r = 0.77$.

Table 2. (continued from preceding page)

Amino acid	Species ^c	No. of functional tRNA genes ^a				Anticodon ^b		Codon specificity ^d	Relative tRNA gene copy no.		Codon usage	
		Ps	Pr			tDNA	tRNA		Ps	Pr	Ps	Pr
Leu	Leu4	6	...	2	...	TAA	UAA	UUA:UUG	0.029	0.014	0.002	0.002
	Leu5	4	...	3	...	TAG	UAG	CUA:CUG	0.019	0.021	0.005	0.005
	Leu6	0	...	0	...	GAG	GAG	CUC*	0	0	0.023	0.022
Lys	Lys1	4	6	4	8	CTT	CUU	AAG	0.019	0.028	0.040	0.039
	Lys2	2	...	4	...	TTT	UUU	AAA:AAG	0.009	0.028	0.007	0.010
Met	Met1	17	17	7	7	CAT	CAU	AUG	0.081	0.050	0.022	0.024
Phe	Phe1	3	3	4	8	GAA	GAA	UUC:UUU	0.014	0.028	0.026	0.027
	Phe2	0	...	4	...	AAA	IAA	UUU:UUC	0	0.028	0.007	0.009
Pro	Pro1	5	8	3	5	AGG	IGG	CCU:[CCC]:CCA	0.024	0.021	0.009	0.009
	Pro2	1	...	1	...	CGG	CGG	CCG	0.005	0.007	0.021	0.018
	Pro3	2	...	1	...	TGG	UGG	CCA:CCG	0.009	0.007	0.009	0.008
	Pro4	0	...	0	...	GGG	GGG	CCC*	0	0	0.013	0.011
Ser	Ser1	1	10	2	8	AGA	IGA	UCU:[UCC]:UCG	0.005	0.014	0.007	0.008
	Ser2	4	...	2	...	CGA	CGA	UCG	0.019	0.014	0.024	0.023
	Ser3	2	...	0	...	GCT	GCU	AGC:[AGU]	0.009	0	0.021	0.019
	Ser4	3	...	4	...	TGA	UGA	UCA:UCG	0.014	0.028	0.006	0.007
	Ser5	0	...	0	...	GGA	GGA	UCC*	0	0	0.013	0.013
	Ser6	0	...	0	...	ACT	ACU	AGU*	0	0	0.008	0.009
Thr	Thr1	2	8	2	6	AGT	IGU	ACU:ACA: [ACG]	0.009	0.014	0.009	0.009
	Thr2	2	...	0	...	CGT	CGU	ACC	0.009	0	0.015	0.014
	Thr3	4	...	4	...	TGT	UGU	ACA:ACG	0.019	0.028	0.007	0.008
	Thr4	0	...	0	...	CGT	CGU	ACG*	0	0	0.026	0.026
Tyr	Tyr1	4	4	2	2	GTA	GUA	UAC:[UAU]	0.019	0.014	0.020	0.020
	Tyr2	0	...	0	...	ATA	AUA	UAU*	0	0	0.004	0.005
Trp	Trp1	1	1	1**	1**	CCA	CCA	UGG	0.005	0.007	0.013	0.012
Val	Val1	1	18	1	10	AAC	IAC	GUU:[GUC]:GUA	0.005	0.007	0.009	0.010
	Val2	8	...	6	...	CAC	CAC	GUG	0.038	0.043	0.035	0.037
	Val3	9	...	3	...	TAC	UAC	GUA:GUG	0.043	0.021	0.004	0.005
	Val4	0	...	0	...	GAC	GAC	GUC*	0	0	0.020	0.019
	Gly4	4	16	0	11	ACC	ACC	GGU	...	...	...	...
pseudo	Trp1	7	...	0	...	CCA	CCA	UGG	...	...	...	...
	Met1	1	...	0	...	CAT	CAU	AUG	...	...	...	...
	Pro2	1	...	0	...	CGG	CGG	CCG	...	...	...	...
	Gly1	1	...	2	...	CCC	CCC	GGG	...	...	...	...
	Lys2	2	...	1	...	TTT	UUU	AAA	...	...	...	...
	Lys1	0	...	1	...	CTT	CUU	AAG	...	...	...	...
	Tyr1	0	...	1	...	GTA	GUA	UAC	...	...	...	...
	???	1	...	6	...	???	???	???	...	...	...	...
	Undetermined	???	14	4	4	???	???	???	...	...	...	...

Sun Fire V250, 2 × 1.28 GHz UltraSPARC IIIi processor machine.

ACKNOWLEDGMENTS

This work was supported by grants to B. M. Tyler from the National Research Initiative of the United States Department of Agriculture Cooperative State Research, Education and Extension Service, grant number 2002-35600-12747, and from the United States National Science Foundation, grant number MCB-0242131, and by funds from the United States Department of Energy Joint Genome Institute and the Virginia Bioinformatics Institute. We thank M. C. Chibucos for careful editing of the manuscript.

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