Supplementary material:

Methodology:

Relative synonymous codon usage (RSCU)

RSCU value of each codon is the observed frequency of that codon divided by the expected frequency for synonymous codons of an amino acid using equal usage as a conjecture.

Thus,

$$RSCU_i = X_i/(1/n \sum_{i=1}^{n} X_i)$$

Where X_i is the occurrence of i^{th} synonymous codon of an *n*-fold degenerate amino acid and *n* may take any one of the values, 1, 2, 3, 4 and 6. RSCU value of 1 indicates even usage of codon and its value lesser than, or greater than one, indicates their uneven use.

Effective Number of Codons (ENC)

ENC is the most widely used estimator of codon usage bias **[21]** and provides the range of codon preferences in a gene. Its value lies among 20 to 61. ENC value of 61indicates equal codon usage for coding an amino acid and, its value is 20 when only single codon codes it. It is estimated as

$$N_c = 2 + {}^{9}/_{\bar{F}_2} + {}^{1}/_{\bar{F}_3} + {}^{5}/_{\bar{F}_4} + {}^{3}/_{\bar{F}_6}$$

Amino acid having multiple synonymous codons is equivalent to a locus, having that number of alleles. If synonymous codons encoding an amino acid are present in equal frequencies then it represents minimum homozygosity. Here $\bar{F}_k$ is the average homozygosity of all k-fold degenerate amino acids.

Table 1: Comparison of genome and CDSs characteristics in six different *Plasmodium* species

Species	Chromosomes	Genome size(Mb)	CDS[a] Count	Genomic GC%	CDS[b] GC%	CDS[c] GC1%	CDS[d] GC2%	[e]CDS GC3%	ENC[f]
P.falciparum	14	23.3	5523	19.41	23.78	31.75	22.19	17.4	37.89
P.vivax	14	27.01	5435	42.3	46.21	46.96	35.18	56.49	55.54
P.knowlesi	14	23.46	5197	37.5	40.19	42.98	32.18	45.39	55.28
P.yoelii	14	23.12	7724	24.69	24.22	31.9	24.12	16.56	38.16
P.chabaudi	14	18.83	251	24.33	26.42	34.01	26.34	18.91	39.71
P.berghei	14	18.52	4904	23.71	23.73	30.86	23.35	16.97	38.61

[a] Coding sequence; [b] Percentage of GC in CDS; [c] Percentage of GC at first site of codon in CDS; [d] Percentage of GC at second site of codon in CDS; [e] Percentage of GC at third site of codon in CDS; ENC[f] is effective number of codons

Table 2: Characterization of synonymous codon usage in the coding sequences of six *Plasmodium* species

		P.falciparum			***P. vivax***			***P.knowlesi***		
Amino Acids	**Codons**	**Codon count**	**RSCU**	**tRNA**	**Codon count**	**RSCU**	**tRNA**	**Codon count**	**RSCU**	**tRNA**
Ala	GCA	34793	1.7	1	50681	1.03	1	48553	1.39	1
	GCC	8765	0.43	0	60369	1.22	0	33951	0.97	0
	GCG	4490	0.22	1	54869	1.11	1	26736	0.77	1
	GCT	33929	1.66	1	31847	0.64	1	30398	0.87	1
Arg	AGA	66359	3.62	1	46194	1.5	1	55508	2.05	1
	AGG	18131	0.99	1	65474	2.12	1	51534	1.9	1
	CGA	10074	0.55	1	17759	0.58	1	17631	0.65	1
	CGC	1786	0.1	0	23601	0.77	0	13147	0.49	0
	CGG	1184	0.06	0	21295	0.69	0	11553	0.43	0
	CGT	12491	0.68	1	10612	0.34	1	13282	0.49	1
Asn	AAC	83114	0.28	1	144197	1.06	1	129407	0.87	1
	AAT	512727	1.72	0	128740	0.94	0	169295	1.13	0
Asp	GAC	36349	0.27	1	109940	1.07	0	84445	0.81	1
	GAT	231759	1.73	0	96160	0.93	0	123089	1.19	0
Cys	TGC	9692	0.26	1	45211	1.3	1	33619	0.96	1
	TGT	63879	1.74	1	24272	0.7	0	36445	1.04	0
Gln	CAA	99479	1.73	1	55907	0.91	0	63750	1.1	1
	CAG	15366	0.27	1	67594	1.09	1	52433	0.9	1
Glu	GAA	253145	1.71	11	152141	1.05	1	175380	1.26	1
	GAG	42934	0.29	1	137884	0.95	1	102750	0.74	1
Gly	GGA	51636	1.76	1	63440	1.02	1	75242	1.49	1
	GGC	5576	0.19	1	62213	1	1	31260	0.62	1
	GGG	11543	0.39	0	83649	1.35	0	49851	0.98	0
	GGT	48919	1.66	0	38612	0.62	0	46208	0.91	0
His	CAC	14471	0.29	1	63638	1.24	1	44525	0.95	1
	CAT	85842	1.71	0	38668	0.76	0	49048	1.05	0

Ile	ATA	209126	1.63	1	65047	0.92	1	83324	1.07	1
	ATC	26171	0.2	0	66006	0.93	0	55344	0.71	0
	ATT	150010	1.17	2	80780	1.14	1	93999	1.21	1
Leu	CTA	25204	0.48	1	36455	0.71	1	38515	1	0
	CTC	7516	0.14	0	57220	1.11	0	39138	1.02	0
	CTG	6245	0.12	1	76151	1.48	1	46647	1.21	1
	CTT	36391	0.69	1	29225	0.57	0	37960	0.99	1
	TTA	196854	3.74	1	47446	0.92	1	1592	0.04	1
	TTG	44005	0.84	1	63264	1.23	1	66832	1.74	1
Lys	AAA	397696	1.63	6	177216	1.04	1	197893	1.14	1
	AAG	89601	0.37	1	163068	0.96	1	149760	0.86	1
Phe	TTC	29733	0.33	2	74227	0.92	1	65341	0.84	1
	TTT	151980	1.67	0	86793	1.08	0	91095	1.16	0
Pro	CCA	37574	1.82	1	34012	1	1	36283	1.32	1
	CCC	8603	0.42	1	56071	1.65	0	32679	1.19	0
	CCG	3955	0.19	1	24870	0.73	0	14802	0.54	1
	CCT	32522	1.57	1	20822	0.61	0	25939	0.95	0
Ser	AGC	16272	0.37	1	96379	1.9	0	57647	1.23	1
	AGT	84758	1.92	0	56349	1.11	0	66663	1.42	0
	TCA	68951	1.56	2	27116	0.53	1	35969	0.77	1
	TCC	21347	0.48	0	59906	1.18	0	53484	1.14	0
	TCG	12549	0.28	1	35543	0.7	2	28247	0.6	1
	TCT	61057	1.38	1	28859	0.57	1	38920	0.83	1
Thr	ACA	90367	2.12	3	34672	0.85	0	53934	1.28	1
	ACC	19983	0.47	1	54590	1.35	0	43979	1.04	0
	ACG	15715	0.37	1	48455	1.19	1	38397	0.91	1
	ACT	44087	1.04	1	24603	0.61	1	32898	0.78	1
Tyr	TAC	26009	0.22	1	94635	1.25	1	80054	1.06	0
	TAT	211010	1.78	1	57169	0.75	0	71550	0.94	0
Val	GTA	65044	1.64	2	35989	0.73	0	47021	1.01	1
	GTC	10114	0.26	2	39013	0.79	0	29212	0.63	0
	GTG	19968	0.5	1	81891	1.66	1	61303	1.32	1
	GTT	63088	1.6	1	40584	0.82	0	47923	1.03	1
Met	ATG	91194	1	3	76190	1	1	83822	1	2
Trp	TGG	20693	1	1	23413	1	1	24229	1	1

		P.yoelii			***P.chabaudi***			***P.berghei***		
Amino Acids	**Codons**	**Codon count**	**RSCU**	**tRNA**	**Codon count**	**RSCU**	**tRNA**	**Codon count**	**RSCU**	**tRNA**
Ala	GCA	38797	1.94	1	2094	1.85	1	36711	1.93	1
	GCC	7520	0.38	0	517	0.46	0	7287	0.38	0
	GCG	3942	0.2	1	266	0.23	1	4124	0.22	1
	GCT	29848	1.49	0	1662	1.46	1	27776	1.46	1
Arg	AGA	50385	3.55	1	2336	3.38	1	50086	3.47	1
	AGG	9787	0.69	1	516	0.75	1	11349	0.79	1
	CGA	13429	0.95	1	722	1.05	1	14219	0.99	1
	CGC	1801	0.13	0	86	0.12	0	1944	0.13	0
	CGG	1956	0.14	0	95	0.14	0	1867	0.13	0
	CGT	7804	0.55	1	389	0.56	1	7043	0.49	1
Asn	AAC	67544	0.31	2	3238	0.35	2	72949	0.33	2
	AAT	366255	1.69	0	15433	1.65	0	375658	1.67	0
Asp	GAC	29987	0.3	1	1738	0.37	2	30829	0.33	2
	GAT	168558	1.7	0	7651	1.63	0	158791	1.67	0
Cys	TGC	12303	0.42	1	676	0.49	1	15102	0.52	1
	TGT	46259	1.58	0	2094	1.51	0	42597	1.48	0
Gln	CAA	82349	1.77	1	4176	1.73	1	81299	1.76	1
	CAG	10489	0.23	1	649	0.27	1	10913	0.24	1
Glu	GAA	209247	1.75	0	9331	1.69	2	209975	1.76	2
	GAG	29302	0.25	1	1696	0.31	1	29097	0.24	1
Gly	GGA	52641	1.92	1	2805	1.9	1	49605	1.95	1
	GGC	8690	0.32	1	571	0.39	1	8422	0.33	1
	GGG	13505	0.49	0	735	0.5	0	14675	0.58	0
	GGT	34755	1.27	0	1789	1.21	0	29052	1.14	0
His	CAC	10914	0.34	1	597	0.38	1	11438	0.35	1
	CAT	52962	1.66	0	2549	1.62	0	54047	1.65	0
Ile	ATA	167453	1.54	1	7381	1.54	1	179731	1.56	1
	ATC	22147	0.2	2	1135	0.24	0	22567	0.2	0
	ATT	135952	1.25	1	5886	1.23	1	142256	1.24	1
Leu	CTA	23276	0.53	1	1212	0.58	1	24206	0.54	1
	CTC	5832	0.13	0	298	0.14	0	5712	0.13	0
	CTG	4518	0.1	1	264	0.13	1	4959	0.11	1
	CTT	30415	0.69	1	1572	0.76	1	30297	0.67	1

	TTA	162034	3.7	1	7339	3.53	1	164738	3.67	1
	TTG	36870	0.84	2	1780	0.86	1	39492	0.88	1
Lys	AAA	340805	1.75	2	14725	1.67	2	352969	1.75	2
	AAG	48673	0.25	1	2944	0.33	1	49891	0.25	1
Phe	TTC	20237	0.25	1	1003	0.28	2	20669	0.25	2
	TTT	140626	1.75	0	6262	1.72	0	145182	1.75	0
Pro	CCA	38708	2.09	1	2287	2.1	1	36227	2.06	1
	CCC	8342	0.45	0	557	0.51	0	7845	0.45	0
	CCG	3511	0.19	1	217	0.2	1	3543	0.2	1
	CCT	23398	1.27	0	1290	1.19	1	22888	1.3	1
Ser	AGC	21780	0.54	2	1275	0.64	2	22965	0.58	2
	AGT	68288	1.69	0	3458	1.74	0	65868	1.66	0
	TCA	66893	1.66	1	3357	1.69	1	66547	1.68	1
	TCC	13641	0.34	0	798	0.4	0	15010	0.38	0
	TCG	13718	0.34	1	711	0.36	1	13543	0.34	1
	TCT	57621	1.43	0	2340	1.18	1	54037	1.36	1
Thr	ACA	75610	2.1	3	3733	2.09	1	75399	2.12	1
	ACC	14101	0.39	0	757	0.42	0	12922	0.36	0
	ACG	9230	0.26	0	526	0.29	1	9233	0.26	1
	ACT	44993	1.25	1	2137	1.2	1	44464	1.25	1
Tyr	TAC	21541	0.24	0	1166	0.28	2	24271	0.26	2
	TAT	159855	1.76	0	7266	1.72	0	164340	1.74	0
Val	GTA	47397	1.54	2	2278	1.5	1	45801	1.53	1
	GTC	7884	0.26	0	437	0.29	0	7768	0.26	0
	GTG	12946	0.42	1	652	0.43	1	12518	0.42	1
	GTT	54898	1.78	1	2688	1.78	1	53449	1.79	1
Met	ATG	64260	1	3	3152	1	3	63868	1	3
Trp	TGG	17135	1	1	873	1	1	16847	1	1