Supplementary Material online:
A Generalized Mechanistic Model for double and triple codon substitution

Maryam Zaheri[1,2,*], Linda Dib[1,2,*] and Nicolas Salamin[1,2]

[1]Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland

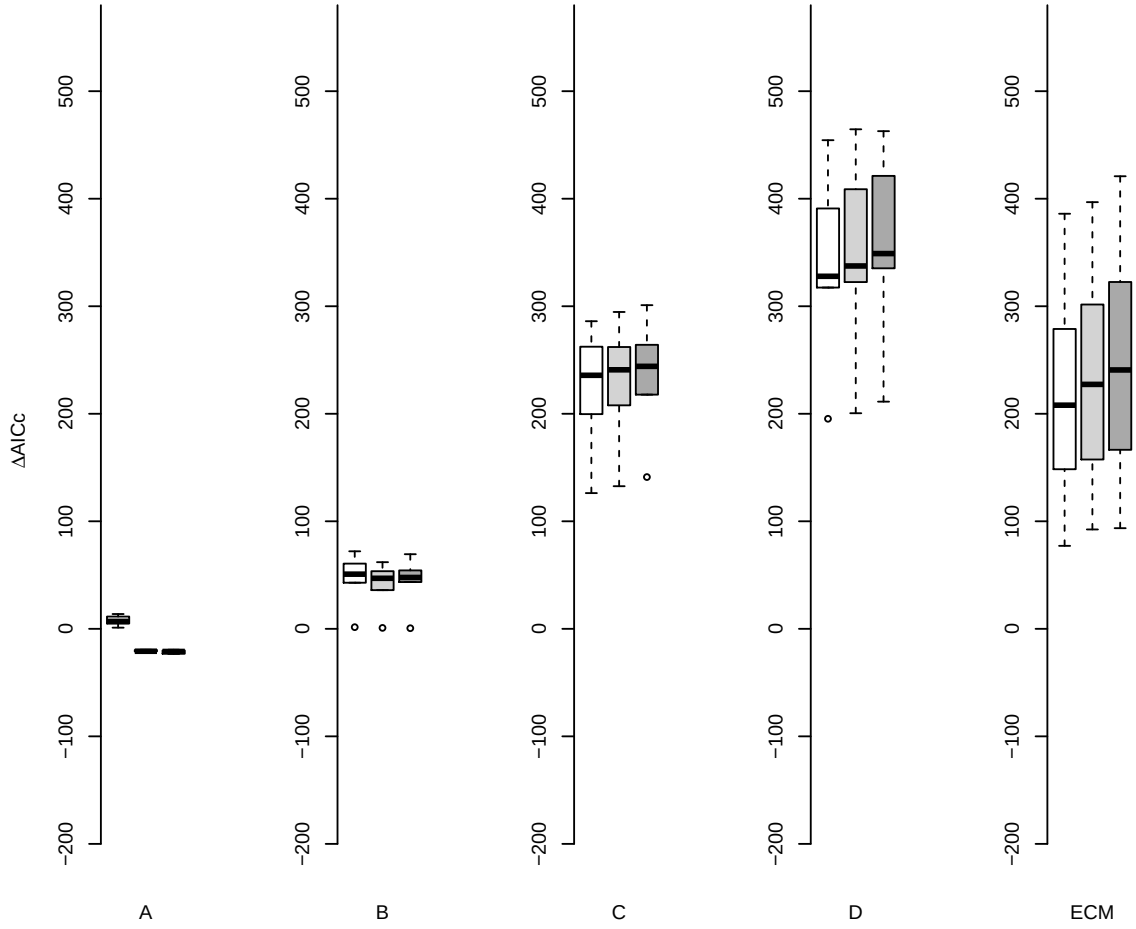
[2]Swiss Institute of Bioinformatics, Quartier Sorge, 1015 Lausanne, Switzerland

[*]co-first authors

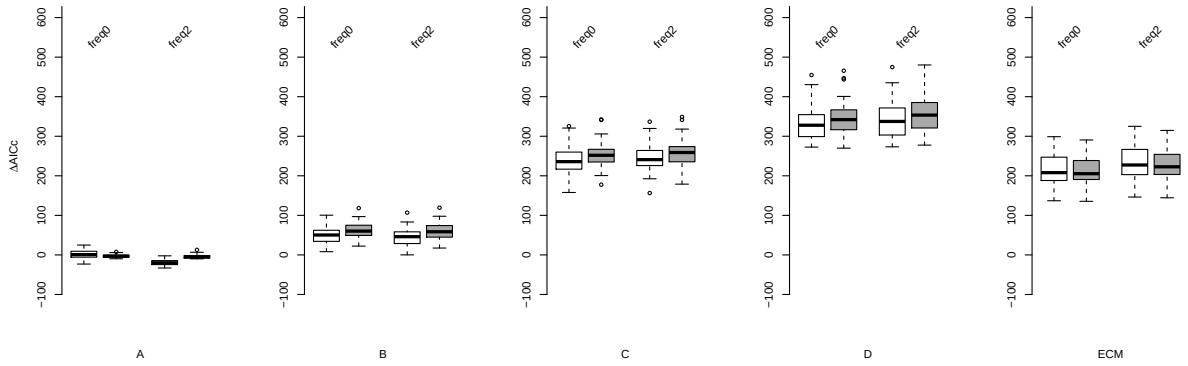
Corresponding author: Nicolas Salamin, Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland; tel: +41 21 692 4154; fax: +41 21 692 4265; email: nicolas.salamin@unil.ch

Keywords: codon models, phylogenetics, multiple substitutions, positive selection, Markov model, Kronecker product

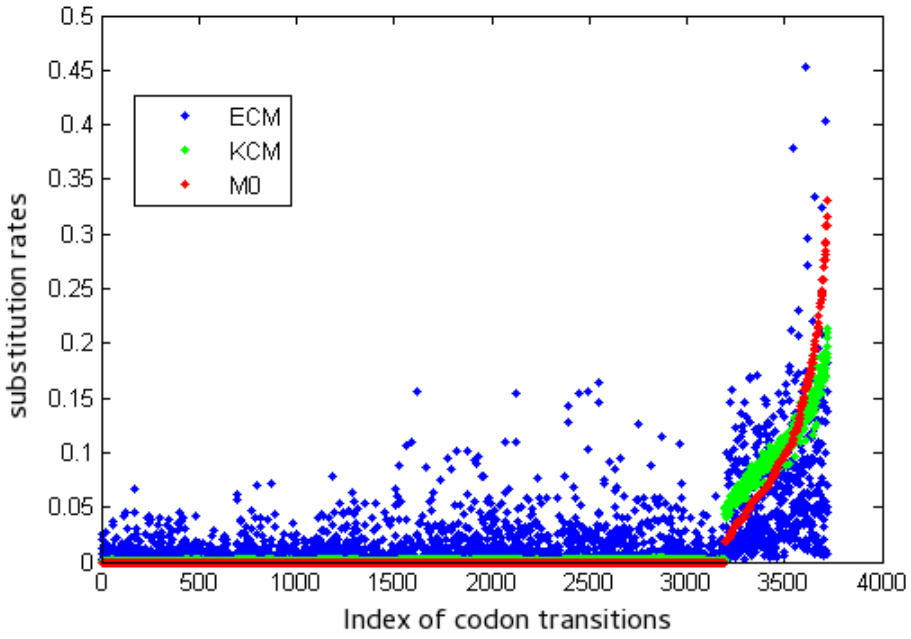
Running head: Generalized codon model



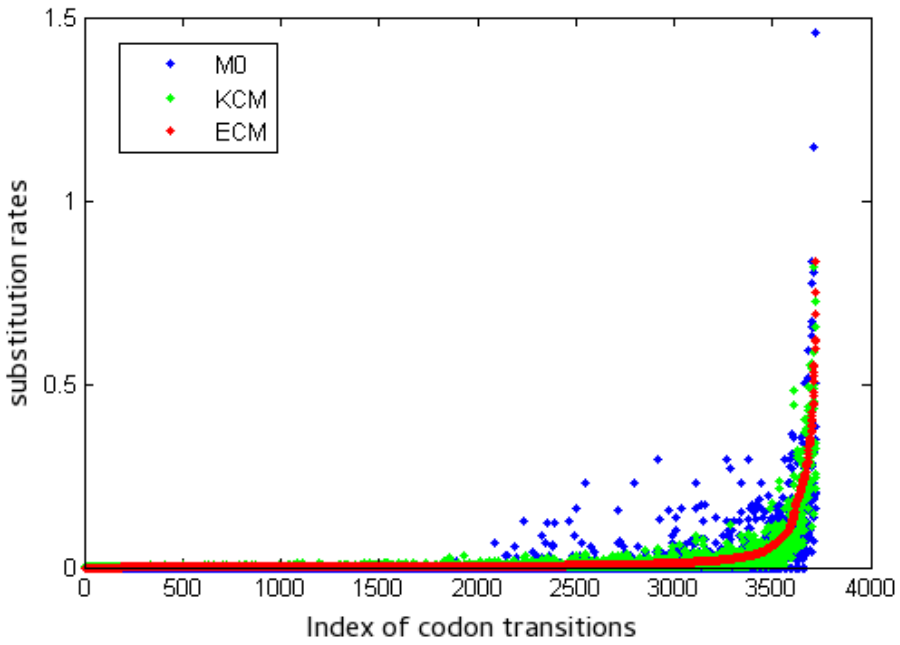
Supplementary fig. 1: Delta AICc plots comparing the performance of the KCM_{19x} model to *M0* model on 5 simulated data sets. Here, we compared the models using three frequency modes: $F1/61$ (white), $F3 \times 4$ (light grey) and $F61$ (dark grey) as mentionned in Yang and Bielawski (2000).



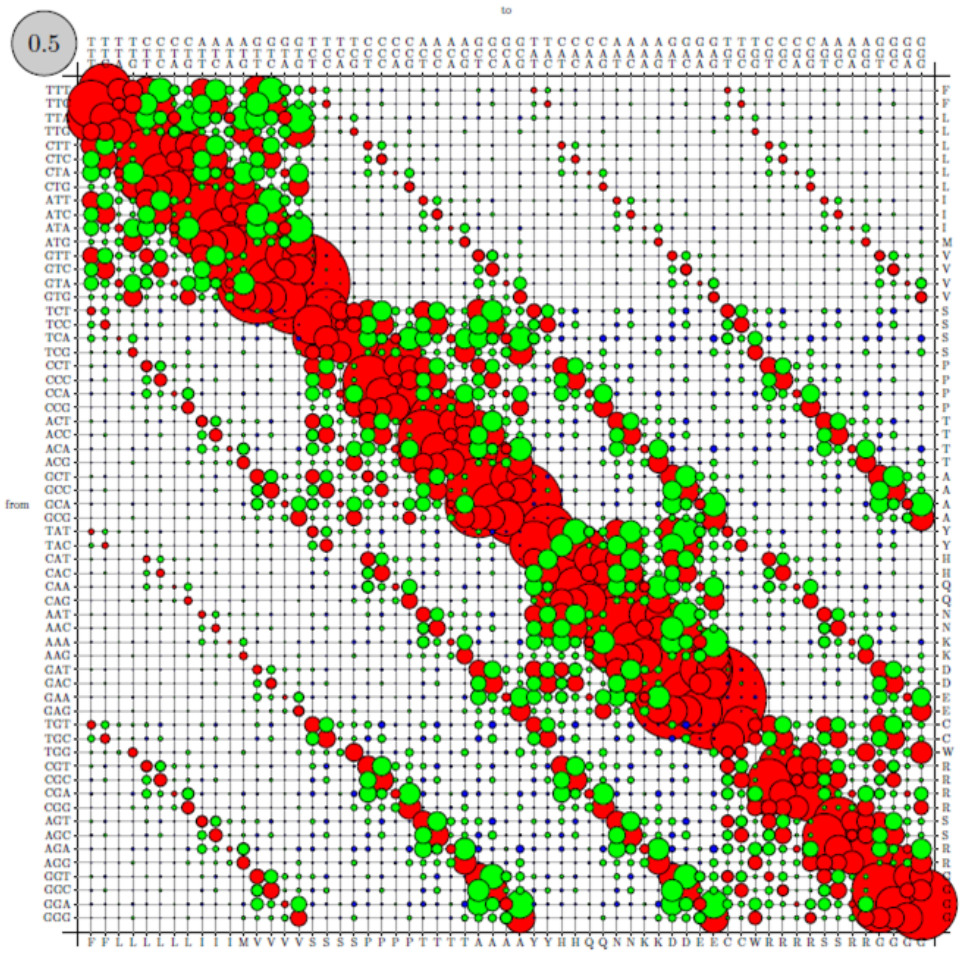
Supplementary fig. 2: Delta AICc plots comparing the performance of the KCM_{7x} and KCM_{19x} models to *M0* model on 5 simulated data sets for ω factor $= 1$. We compared the models using two frequency modes: the first one where all frequencies are equal ($F1/61$) and the second where we used the products of the observed nucleotide frequencies at each of the 3 codon positions ($F3 \times 4$; Yang and Bielawski, 2000).



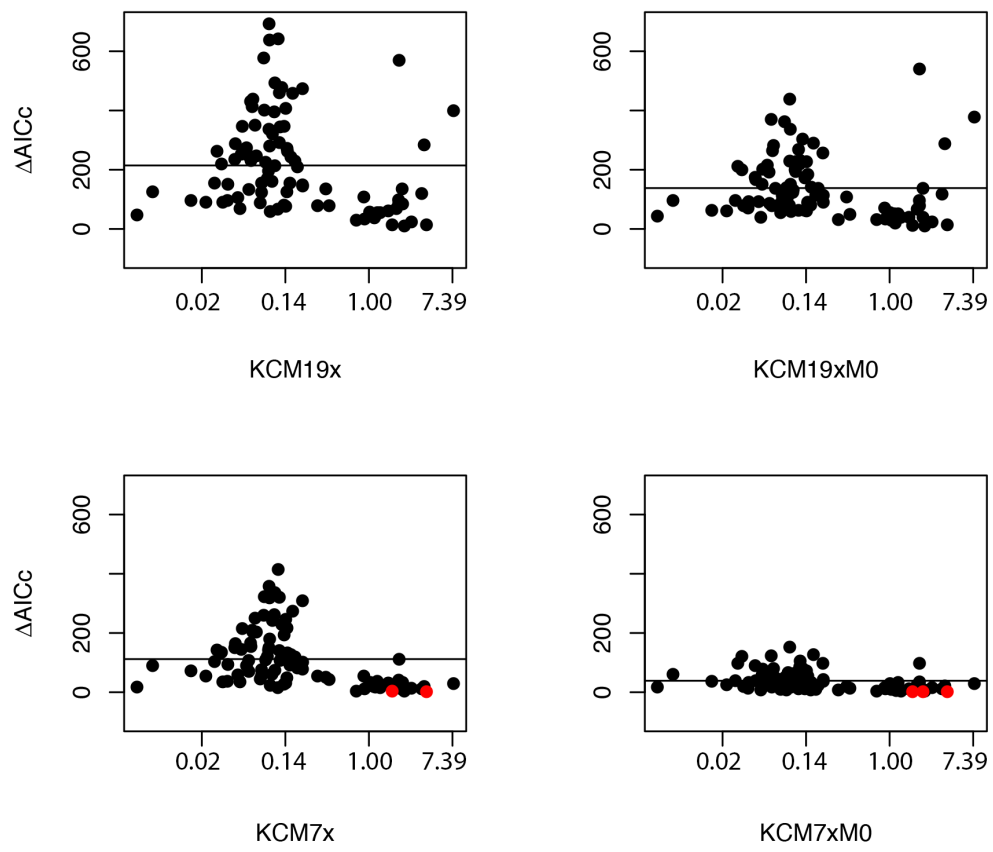
Supplementary fig. 3: Plot of substitution rates per codon of KCM_{19x}, *ECM* and *M0* models for data simulated under the *M0* model. The x-axis is the index of the 3,660 rates of transition between codons obtained from the rate matrices estimated by the different models, while the y-axis is the rate of substitution per codon. The substitution rates from the *M0* model is used as the reference distribution. The median of the 50 simulations for each entity of the 61×61 rate matrix are plotted for *M0* (red), KCM_{19x} (green) and *ECM* (blue) models. The simulations are realized under the *M0* model with $\omega = 1$. The points are sorted according to the entities of the *M0* substitution rate matrix. The Euclidean distance between KCM_{19x} substitution rates and the *M0* ones was 1.005, while for *ECM*the Euclidian distance with *M0* rates was 2.281.



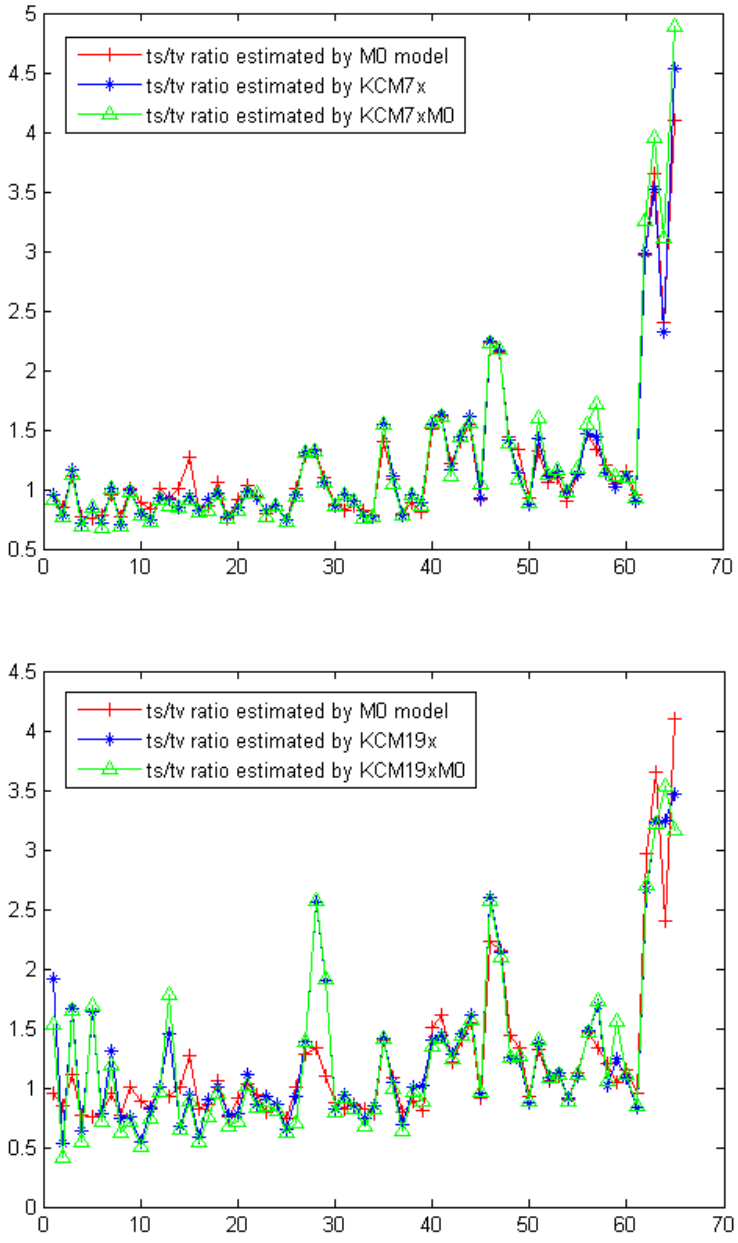
Supplementary fig. 4: Plot of substitution rates per codon of KCM_{19x} *ECM* and *M0* models for data simulated under the *ECM* model. The x-axis is the index of the 3,660 rates of transition between codons obtained from the rate matrices estimated by the different models, while the y-axis is the rate of substitution per codon. The substitution rates from the *ECM* model is used as the reference distribution. The median of the 50 simulations for each entity of the 61×61 rate matrix are plotted for *ECM* (red), KCM_{19x} (green) and *M0* (blue). The simulations are realized under the *ECM* model with ω factor $= 1$. The points are sorted according to the entities of the *ECM* substitution rate matrix. The Euclidean distance between KCM_{19x} substitution rates and the *ECM* ones was 1.812, while the Euclidean distance between *M0* substitution rates and the *ECM* ones was 2.789.



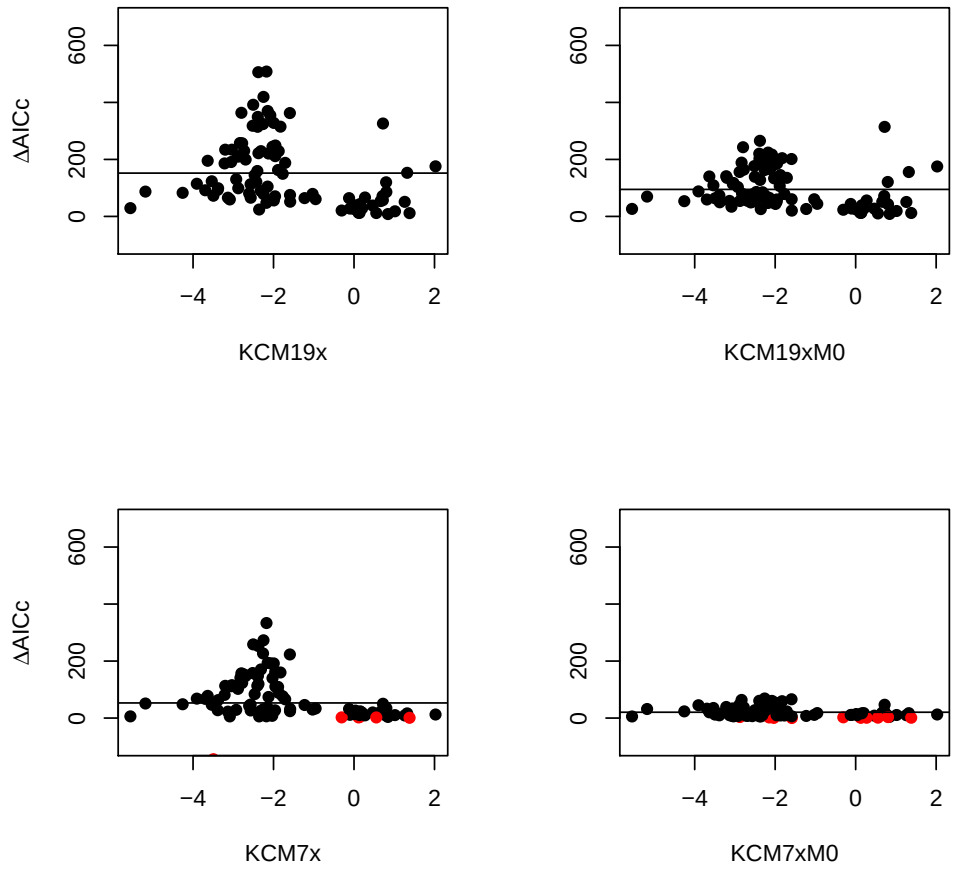
Supplementary fig. 5: Bubble plot of the KCM_{19x} model for the β-*globin* data sets. Single, double and triple nucleotide substitutions are in red, green and blue respectively. Codons are according to Urbina et al (2006).



Supplementary fig. 6: Delta AICc plots comparing the performance of the *M0* model to all variants of the *KCM* model on 100 empirical data sets randomly selected from the Selectome data base. For each plot, a black horizontal line is draw for the mean delta AICc value of the empirical dataset. Note that for this analysis we considered a frequency vector calculated using the products of the observed nucleotide frequencies at each of the 3 codon positions ($F3 \times 4$ Yang and Bielawski, 2000). Empirical data sets with delta AICc < 4 are shown in red.



Supplementary fig. 7: Estimation of the average rate of transition and transversion for the empirical data sets selected from the Selectome database. We only used the 65 data sets that did not contain gaps to create this plot. The rates are estimated from the nucleotide substitution matrix (the rates for the three matrices of KCM_{19x} are averaged) based on the formula described in Yang (2006).



Supplementary fig. 8: Delta AICc plots comparing the performance of the *M0* model to all variants of the *KCM* model on 100 empirical data sets randomly selected from the Selectome data base. For each plot, a black horizontal line is draw for the mean delta AICc value of the empirical dataset. Note that for this analysis we considered a frequency vector with equal codon frequencies ($F1/61$ Yang and Bielawski, 2000). Empirical data sets with delta AICc < 4 are shown in red.

Supplementary tables

Supplementary table 1: Comparison of the estimation of the ω factors for data simulated with 150 and 3500 codons. The median and mean ω estimated for different alignment lengths is reported for simulations A, B and ECM with factors on the ω parameter of 0.5 and 2.0.

ω factor	Simulations	Mean		Variance	
		150 codons	3500 codons	150 codons	3500 codons
0.5	A	0.519	0.514	1.606e-2	1.063e-3
	B	0.395	0.369	7.514e-3	3.926e-4
	ECM	0.042	0.035	1.149e-4	1.954e-6
2.0	A	2.614	2.206	3.297e-1	1.504e-2
	B	1.580	1.500	4.351e-1	1.792e-2
	ECM	0.135	0.130	8.091e-4	4.271e-5

Supplementary table 2: Mean, variance, minimum and maximum total branch lengths for a whole alignment (450 codons) for the simulated data sets based on the settings of simulation A with ω factor set to 0.1, 0.5, 1, 2 and 10. The large variance obtained for ω factor = 0.1 is due to a single simulation leading to large divergence between sequences (i.e. the maximum value of 110.808).

ω factor	Total branch lengths			
	Mean	Variance	Minimum	Maximum
0.1	54.806	176.515	36.651	110.808
0.5	41.582	13.836	36.397	54.520
1.0	38.285	8.503	32.229	47.488
2.0	36.981	5.490	31.759	42.425
10.0	38.365	6.484	32.723	42.823

Supplementary table 3: List of the 100 empirical data sets selected from the Selectome database. The value of ω was estimated with the *M0* model.

Family	ω	Number of species	Alignment length
ENSGT00390000007638.Euteleostomi.001	8.66745	10	229
ENSGT00530000064869.Euteleostomi.001	6.462	8	98
ENSGT00390000016329.Euteleostomi.001	3.923	12	88
ENSGT00400000024605.Euteleostomi.001	2.744	9	163
ENSGT00390000016066.Euteleostomi.001	2.656	14	178
ENSGT00410000029170.Euteleostomi.001	2.494	9	62

Continued on next page

Supplementary table 4 – continued from previous page

Family	ω	Number of species	Alignment length
ENSGT00390000018599.Euteleostomi.001	2.408	8	168
ENSGT00680000100237.Euteleostomi.001	2.397	13	404
ENSGT00530000064897.Euteleostomi.001	2.332	9	144
ENSGT00410000026913.Euteleostomi.001	2.313	13	384
ENSGT00680000100326.Euteleostomi.001	2.233	9	105
ENSGT00410000028440.Euteleostomi.001	2.217	8	69
ENSGT00530000065014.Euteleostomi.001	2.198	7	143
ENSGT00530000064466.Euteleostomi.001	2.113	11	64
ENSGT00540000072033.Euteleostomi.001	2.014	23	636
ENSGT00680000100122.Euteleostomi.001	1.933	25	428
ENSGT00680000101349.Euteleostomi.001	1.857	16	166
ENSGT00540000072033.Primates.001	1.794	24	567
ENSGT00530000062931.Primates.005	1.775	28	72
ENSGT00630000089916.Euteleostomi.001	1.612	48	64
ENSGT00680000100179.Euteleostomi.001	1.319	22	401
ENSGT00400000022365.Primates.001	1.226	16	133
ENSGT00390000003272.Euteleostomi.001	1.214	17	287
ENSGT00390000003272.Primates.001	1.211	17	273
ENSGT00650000093893.Euteleostomi.001	1.187	19	139
ENSGT00680000100090.Euteleostomi.001	1.171	41	579
ENSGT00390000012484.Primates.001	1.108	33	229
ENSGT00700000104581.Primates.001	1.027	20	491
ENSGT00610000086906.Euteleostomi.001	1.026	17	190
ENSGT00400000022365.Euteleostomi.001	1.007	16	128
ENSGT00530000064497.Primates.001	0.878	20	142
ENSGT00390000012671.Euteleostomi.001	0.451	26	310
ENSGT00700000104246.Primates.002	0.447	27	90
ENSGT00390000001323.Euteleostomi.001	0.384	23	225
ENSGT00390000006080.Euteleostomi.001	0.313	32	847
ENSGT00390000014593.Euteleostomi.001	0.302	25	232
ENSGT00650000093105.Euteleostomi.007	0.286	27	516
ENSGT00390000000252.Euteleostomi.001	0.237	25	268
ENSGT00390000003056.Euteleostomi.001	0.234	18	417
ENSGT00510000047079.Euteleostomi.001	0.228	41	380
ENSGT00440000039958.Euteleostomi.001	0.222	19	518
ENSGT00570000078866.Euteleostomi.003	0.206	30	488
ENSGT00550000074403.Euteleostomi.003	0.202	45	406
ENSGT00390000017791.Euteleostomi.001	0.190	42	204
ENSGT00390000011439.Euteleostomi.001	0.190	37	343
ENSGT00390000002149.Euteleostomi.003	0.184	38	370
ENSGT00390000018632.Euteleostomi.001	0.175	19	912
ENSGT00390000001055.Euteleostomi.001	0.170	47	217
ENSGT00550000075001.Euteleostomi.001	0.170	40	450
ENSGT00530000063224.Euteleostomi.003	0.165	33	595

Continued on next page

Supplementary table 4 – continued from previous page

Family	ω	Number of species	Alignment length
ENSGT00530000062962.Euteleostomi.002	0.162	47	366
ENSGT00390000000377.Euteleostomi.001	0.156	33	824
ENSGT00390000016028.Euteleostomi.006	0.147	39	401
ENSGT00390000011804.Euteleostomi.001	0.142	30	159
ENSGT00680000099596.Euteleostomi.004	0.139	32	635
ENSGT00390000018208.Euteleostomi.001	0.136	26	168
ENSGT00390000010568.Euteleostomi.001	0.135	43	296
ENSGT00550000074304.Euteleostomi.004	0.135	36	541
ENSGT00550000074424.Euteleostomi.001	0.132	46	503
ENSGT00390000004323.Euteleostomi.001	0.131	33	268
ENSGT00390000002563.Euteleostomi.002	0.129	42	593
ENSGT00510000048936.Euteleostomi.001	0.129	23	429
ENSGT00390000008178.Euteleostomi.001	0.128	29	722
ENSGT00630000089834.Euteleostomi.001	0.127	36	936
ENSGT00660000095648.Euteleostomi.001	0.126	18	419
ENSGT00390000004313.Euteleostomi.001	0.121	28	185
ENSGT00680000099772.Euteleostomi.001	0.120	29	389
ENSGT00510000047481.Euteleostomi.001	0.114	36	583
ENSGT00530000063670.Euteleostomi.002	0.113	29	550
ENSGT00510000047499.Euteleostomi.001	0.112	33	461
ENSGT00660000095322.Euteleostomi.002	0.108	40	949
ENSGT00390000001969.Euteleostomi.003	0.106	29	804
ENSGT00600000084107.Euteleostomi.001	0.099	41	853
ENSGT00530000063122.Euteleostomi.003	0.091	34	572
ENSGT00530000063319.Euteleostomi.001	0.077	16	580
ENSGT00530000063473.Euteleostomi.001	0.074	30	608
ENSGT00390000014093.Euteleostomi.002	0.073	36	443
ENSGT00620000087732.Euteleostomi.003	0.066	37	499
ENSGT00550000074477.Euteleostomi.002	0.066	44	522
ENSGT00390000014517.Euteleostomi.001	0.066	33	232
ENSGT00390000016435.Euteleostomi.001	0.065	36	527
ENSGT00390000005712.Euteleostomi.001	0.064	37	638
ENSGT00550000074298.Euteleostomi.001	0.063	35	669
ENSGT00530000063581.Euteleostomi.002	0.062	34	422
ENSGT00390000002779.Euteleostomi.001	0.058	38	516
ENSGT00670000097509.Euteleostomi.001	0.056	33	488
ENSGT00390000016168.Euteleostomi.001	0.052	48	688
ENSGT00390000020883.Euteleostomi.001	0.051	18	669
ENSGT00510000047893.Euteleostomi.001	0.051	29	420
ENSGT00660000095228.Euteleostomi.002	0.047	31	412
ENSGT00390000004653.Euteleostomi.003	0.046	35	277
ENSGT00530000062914.Euteleostomi.003	0.039	40	386
ENSGT00560000076730.Euteleostomi.006	0.038	26	462
ENSGT00560000076885.Euteleostomi.003	0.035	48	302

Continued on next page

Supplementary table 4 – continued from previous page

Family	ω	Number of species	Alignment length
ENSGT00660000095340.Euteleostomi.004	0.029	45	998
ENSGT00650000093277.Euteleostomi.004	0.027	38	311
ENSGT00550000074326.Euteleostomi.004	0.022	41	318
ENSGT00390000004852.Euteleostomi.001	0.014	49	183
ENSGT00660000095549.Euteleostomi.001	0.013	31	428
ENSGT00550000075088.Euteleostomi.001	0.005	41	158