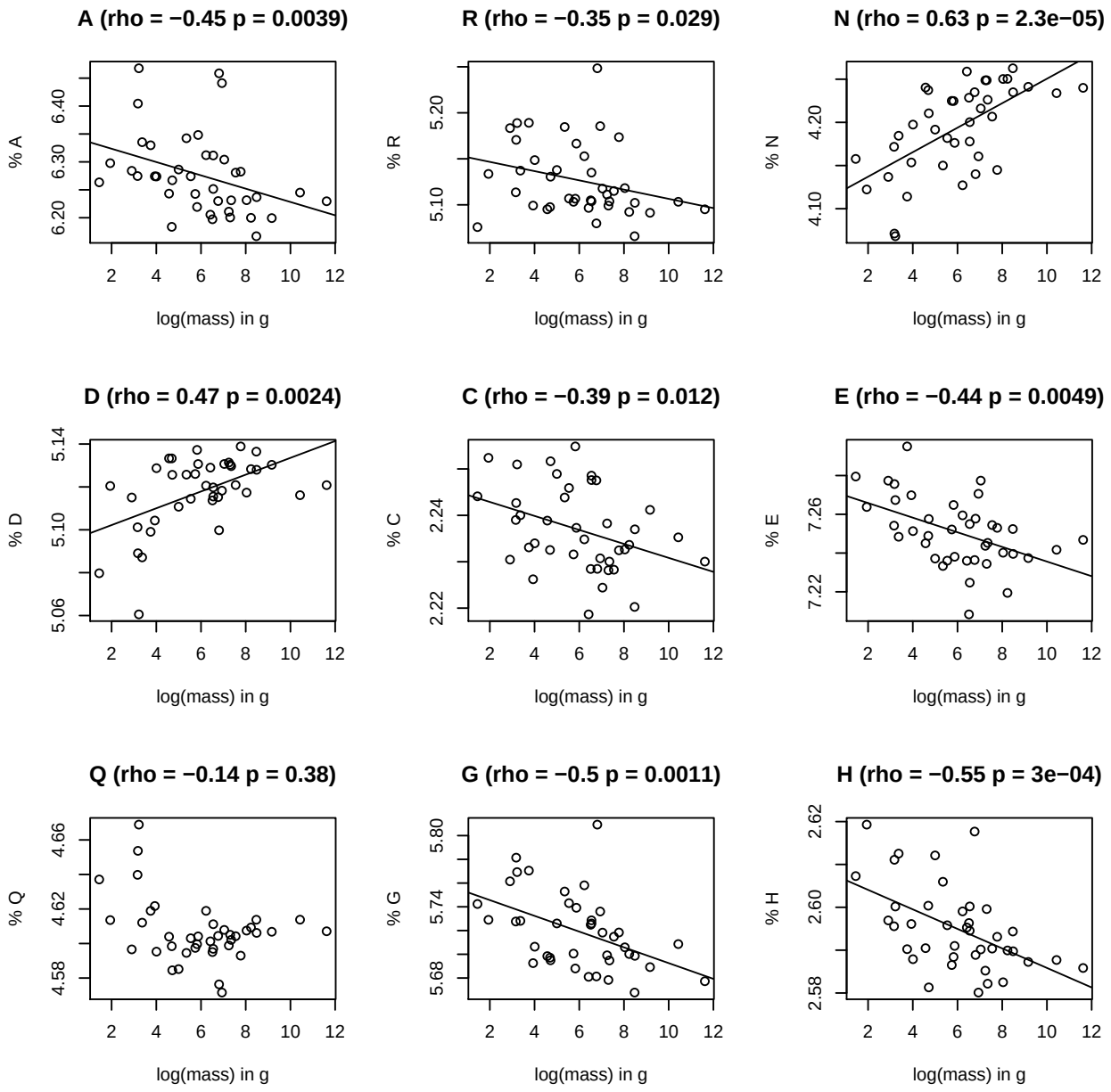
Supplementary file 1

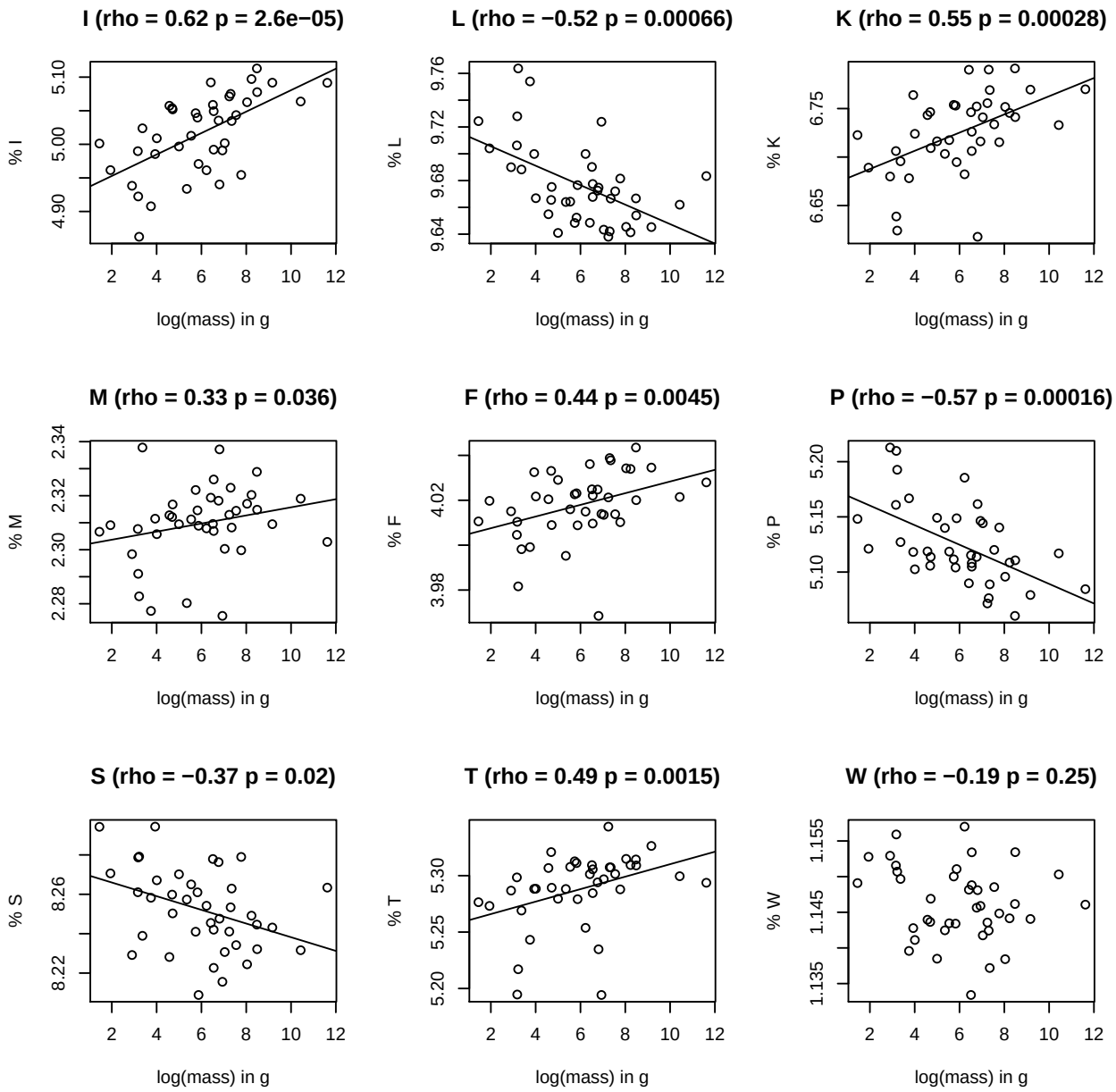
Code	Scientific name	Trivial name
ACACH	*Acanthisitta chloris*	Rifleman
ANAPL	*Anas platyrhynchos*	Mallard (domestic)
ANOCA	*Anolis carolinensis*	Green lizard
APAVI	*Apaloderma vittatum*	Bar-tailed Trogon
APTFO	*Aptenodytes forsteri*	Emperor Penguin
BALRE	*Balearica regulorum*	Grey Crowned Crane
BUCRH	*Buceros rhinoceros*	Rhinoceros Hornbill
CALAN	*Calypte anna*	Anna's Hummingbird
CAPCA	*Antrostomus carolinensis*	Chuck-will's-widow (Nightjar)
CARCR	*Cariama cristata*	Red-legged Seriema
CATAU	*Cathartes aura*	Turkey Vulture
CHAPE	*Chaetura pelagica*	Chimney Swift
CHAVO	*Charadrius vociferus*	Killdeer
CHLUN	*Chlamydotis macqueenii*	Macqueen's Bustard
COLLI	*Columba livia*	Rock Pigeon (domestic)
COLST	*Colius striatus*	Speckled Mousebird
CORBR	*Corvus brachyrhynchos*	American Crow
CUCCA	*Cuculus canorus*	Common Cuckoo
EGRGA	*Egretta garzetta*	Little Egret
EURHE	*Eurypyga helias*	Sunbittern
FALPE	*Falco peregrinus*	Peregrine Falcon
FULGL	*Fulmarus glacialis*	Northern Fulmar
GALGA	*Gallus gallus*	Red Junglefowl (Chicken)
GAVST	*Gavia stellata*	Red-throated Loon
GEOFO	*Geospiza fortis*	Medium Ground-finch
HALAL	*Haliaeetus albicilla*	White-tailed Eagle
HALLE	*Haliaeetus leucocephalus*	Bald eagle
LEPDI	*Leptosomus discolor*	Cuckoo Roller
MANVI	*Manacus vitellinus*	Golden-collared Manakin
MELGA	*Meleagris gallopavo*	Turkey
MELUN	*Melopsittacus undulatus*	Budgerigar
MERNU	*Merops nubicus*	Carmine Bee-eater
MESUN	*Mesitornis unicolor*	Brown Mesite
NESNO	*Nestor notabilis*	Kea
NIPNI	*Nipponia nippon*	Crested Ibis
OPHHO	*Opisthocomus hoazin*	Hoatzin
PELCR	*Pelecanus crispus*	Dalmatian Pelican
PHACA	*Phalacrocorax carbo*	Cormorant
PHALE	*Phaethon lepturus*	White-tailed Tropicbird
PHORU	*Phoenicopterus ruber*	American Flamingo

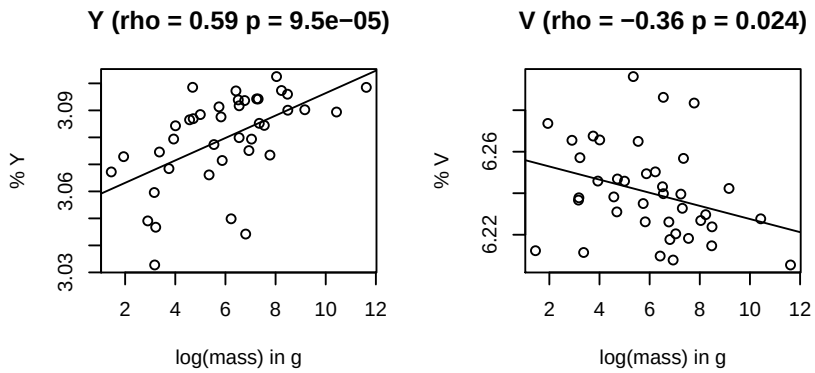
PICPU	*Picoides pubescens*	Downy Woodpecker
PODCR	*Podiceps cristatus*	Great Crested Grebe
PTEGU	*Pterocles gutturalis*	Yellow-throated Sandgrouse
PYGAD	*Pygoscelis adeliae*	Adelie Penguin
STRCA	*Struthio camelus*	Ostrich
TAEGU	*Taeniopygia guttata*	Zebra Finch
TAUER	*Tauraco erythrolophus*	Red-crested Turaco
TINMA	*Tinamus major*	Great Tinamou
TYTAL	*Tyto alba*	Barn Owl

Supplementary file 2

Given the pervasive effects of GC-biased gene converion and correlation between GC content and 2nd codon positions, we might expect that life history is also associated with differences in amino acid usage between species due to the structure of the genetic code. This appears indeed to be the case for a subset of amino acids. Figures represent amino acid usage in percent plotted against log(body mass) in grams, with amino acids labelled in one-letter notation. Rho denotes Spearman's rank correlation coefficient.


A (rho = −0.45 p = 0.0039)
% A
log(mass) in g
R (rho = −0.35 p = 0.029)
% R
log(mass) in g
N (rho = 0.63 p = 2.3e−05)
% N
log(mass) in g
D (rho = 0.47 p = 0.0024)
% D
log(mass) in g
C (rho = −0.39 p = 0.012)
% C
log(mass) in g
E (rho = −0.44 p = 0.0049)
% E
log(mass) in g
Q (rho = −0.14 p = 0.38)
% Q
log(mass) in g
G (rho = −0.5 p = 0.0011)
% G
log(mass) in g
H (rho = −0.55 p = 3e−04)
% H
log(mass) in g


I (rho = 0.62 p = 2.6e−05)
% I
L (rho = −0.52 p = 0.00066)
% L
K (rho = 0.55 p = 0.00028)
% K
M (rho = 0.33 p = 0.036)
% M
F (rho = 0.44 p = 0.0045)
% F
P (rho = −0.57 p = 0.00016)
% P
S (rho = −0.37 p = 0.02)
% S
T (rho = 0.49 p = 0.0015)
% T
W (rho = −0.19 p = 0.25)
% W
log(mass) in g


Y (rho = 0.59 p = 9.5e−05)
% Y
3.03
3.06
3.09
2
4
6
8
10
12
log(mass) in g
V (rho = −0.36 p = 0.024)
% V
6.22
6.26
2
4
6
8
10
12
log(mass) in g

Supplementary file 4

Correlations for 1780 orthologs and their introns are largely robust to control for phylogenetic inertia using a variety of trees obtained from Jarvis et al. (2014). The table below shows correlations between GC at different codon positions as well as intronic GC (GCi) and body mass. The first row represents the uncorrected Spearman's rank correlations and subsequent rows show the correlations after applying PIC (part of the Ape package in R). The 3rd codon position becomes borderline significant after control for the tree based on codon 3. However, this is expected, as the topology of this tree is predicted to be strongly affected by GC3. Thus, using this tree for phylogenetic control of the correlation between GC3 and body mass controls in part for GC3 itself. The already weak intronic correlation becomes non-significant in two cases, but actually becomes stronger in two others, perhaps consistent with noisy intronic GC content.

Spearman's rho	GC all coding	GC1	GC2	GC3	GCi
raw correlation	-0.5869 p = 6.1e-05	-0.5631 p = 0.0001	-0.5639 p = 0.0001	-0.5866 p = 6.2e-05	-0.4411 p = 0.0038
DNA c12 part consensus	-0.4732 p = 0.002	-0.4397 p = 0.0043	-0.4742 p = 0.0019	-0.4777 p = 0.0018	-0.4848 p = 0.001
DNA c123 part best	-0.3331 p = 0.0339	-0.3314 p = 0.0349	-0.3535 p = 0.0239	-0.3505 p = 0.0252	-0.2223 p = 0.162
Codon 3 MRE	-0.2901 p = 0.0661	-0.3232 p = 0.0398	-0.3838 p = 0.0138	-0.272 p = 0.0856	-0.2847 p = 0.0715
Whole genome indel best	-0.4382 p = 0.0045	-0.4134 p = 0.0076	-0.4378 p = 0.0045	-0.4303 p = 0.0053	-0.3233 p = 0.0397
Intron part MRE consensus	-0.4843 p = 0.0015	-0.4519 p = 0.0033	-0.4847 p = 0.0015	-0.4749 p = 0.0019	-0.4817 p = 0.0016
Amino acid Fast Tree	-0.3699 p = 0.0178	-0.3655 p = 0.0193	-0.4186 p = 0.0068	-0.3535 p = 0.0239	-0.3089 p = 0.0499

Supplementary file 4

Alignment	dS vs maturity		GC3 vs maturity	
	correlation	pp	correlation	pp
1	-0.824	0	-0.14	0.28
2	-0.772	0	-0.151	0.27
3	-0.78	0	-0.418	0.069
4	-0.854	0	-0.697	0.0004
5	-0.777	0	-0.588	0.0094
6	-0.757	0	-0.29	0.11
7	-0.962	0	-0.806	0.0054
8	-0.79	0	-0.434	0.036
9	-0.721	0.0004	-0.641	0.005
10	-0.811	0	-0.52	0.042

Results from 10 Coevol chains (see Materials and Methods) show negative correlations through time between age of 1st female sexual maturity (labelled "maturity") and dS, which was used to model trait evolution along the tree. They also show a negative relationship between GC3 and age of 1st female sexual maturity through time. Although not all alignments reached low posterior probabilities, this is consistent with our observation that small-bodied birds with short generation times and large populations tend to have higher GC3.

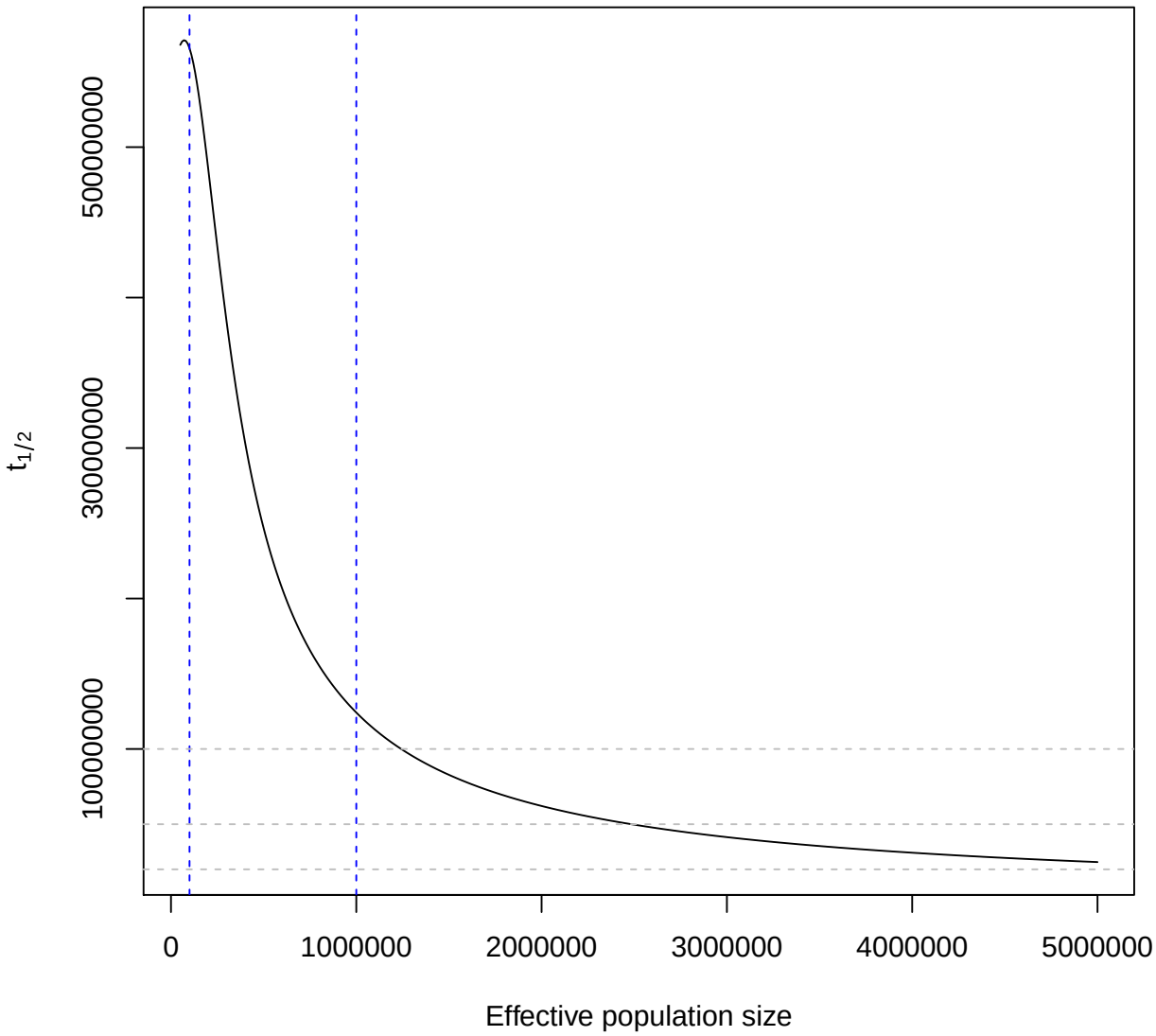
Supplementary file 5

The notion that the number of meioses per yer could influence a species' current GC content hinges on the assumption that equilibrium has yet to be reached. As we demonstrate in Figure 8, this appears to be the case in the majority of species. One might ask whether this is surprising given the number of generations that have passed since the radiation of modern birds. In primates, it has previously been demonstrated that the rate of evolution of base composition, as measured by the rate at which the difference between GC and equilibrium GC is halved (the process half time), is slow: Hundreds of millions of years are required to halve the difference in composition outside of recombination hotspots [14].

To estimate the rate at which base composition evolves in birds, we applied the method of Duret and Arndt (2008) [14] with adjusted parameters. Reports of bird effective population sizes often vary between around 100,000 and 1 million. The gBGC coefficient s has been estimated to be $1e-6$ in chicken [38], while the mutation rate is of the order of $1.5e-9$ [42] and the average bird recombination rate is around 3cM/Mb [41]. We assumed the relative rates of weak to strong and strong to weak mutation to be similar to patterns observed in mammals [14]. As N_e has a strong impact on the inferred process half time (see Figure) we might examine a range of effective population sizes.

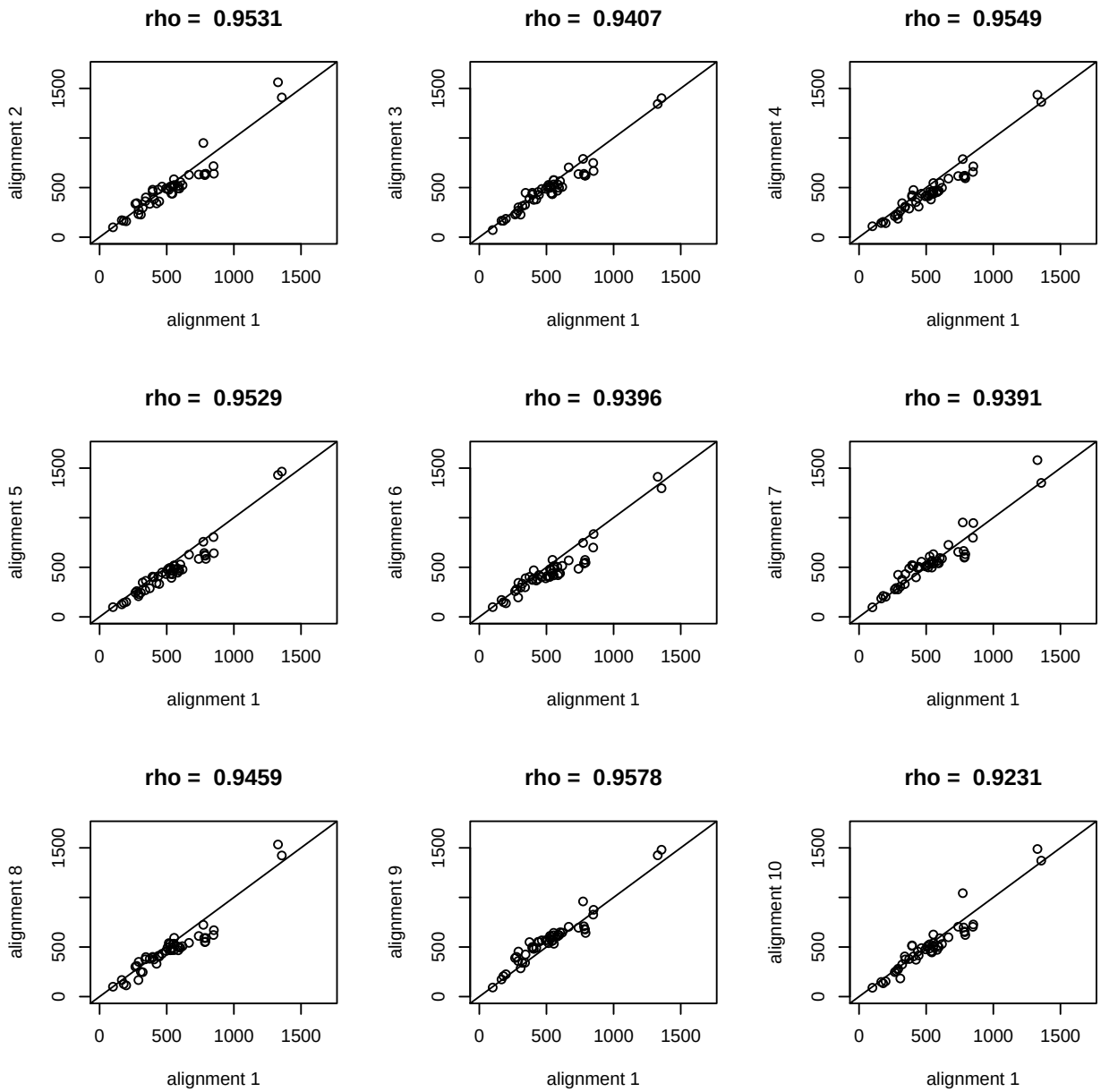
If we for instance consider an effective population size of 100,000, we obtain a process half time of 565,739,002 generations. Meanwhile, an effective population of 1,000,000 gives a half time of 124,218,180 generations. Assuming avian generation times of 2-5 years and modern birds having radiated about 100 Mya [33] (i.e. 20M-50M generations ago), these time scales are too short to have reduced the difference between ancestral and equilibrium compositions by half. It is therefore plausible that our observation that GC3 is smaller than GC3* for the majority of species is explained by the fact that the rate of compositional evolution is slow and insufficient time has passed for equilibrium to have been reached.

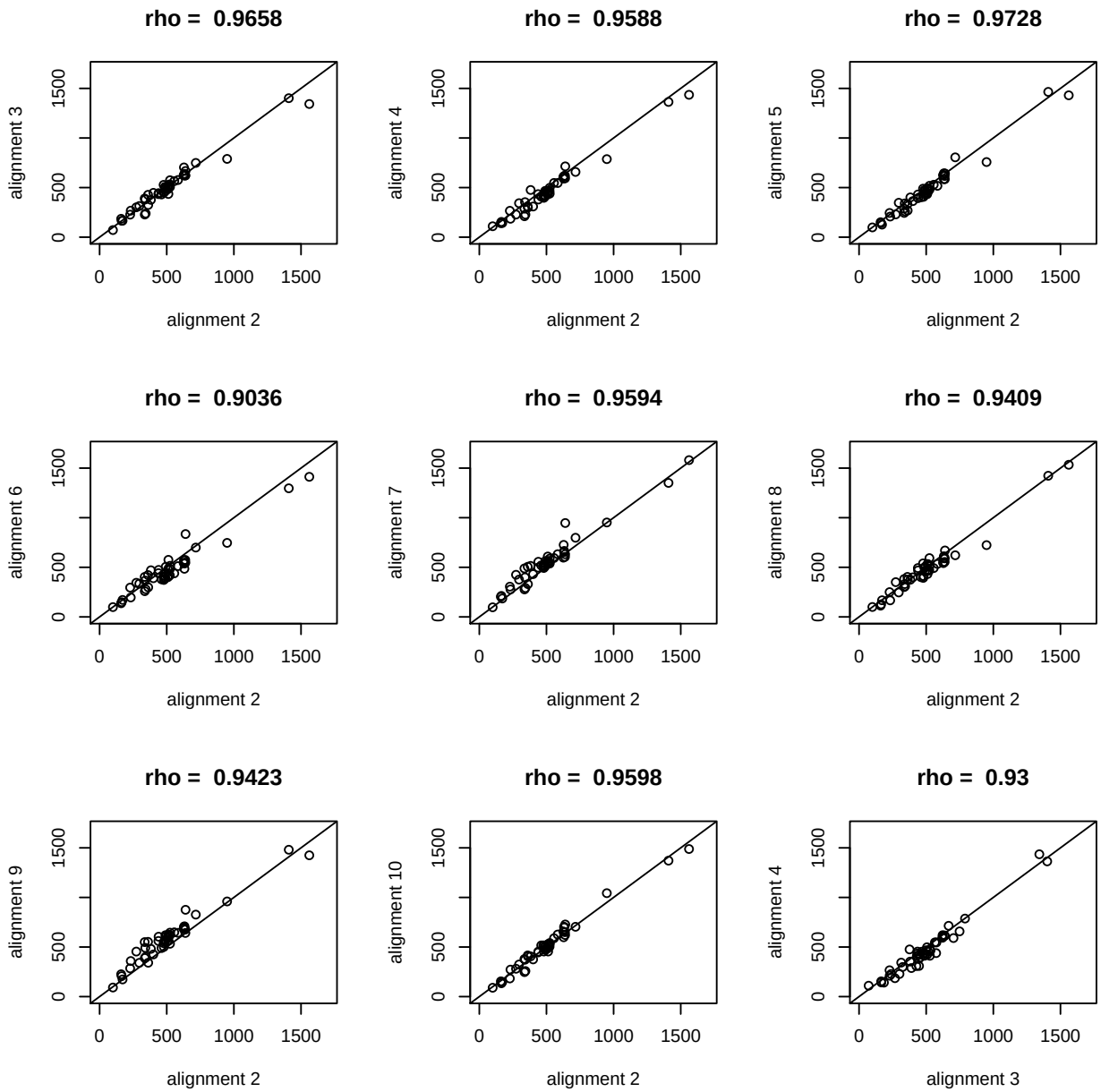
Figure S5: The rate at which the difference between GC3 and GC3* halves, $t_{1/2}$ depends on N_e. The blue lines delineate the range from 100,000 to 1,000,000. The grey lines indicate 100M, 50M and 20M generations, respectively.

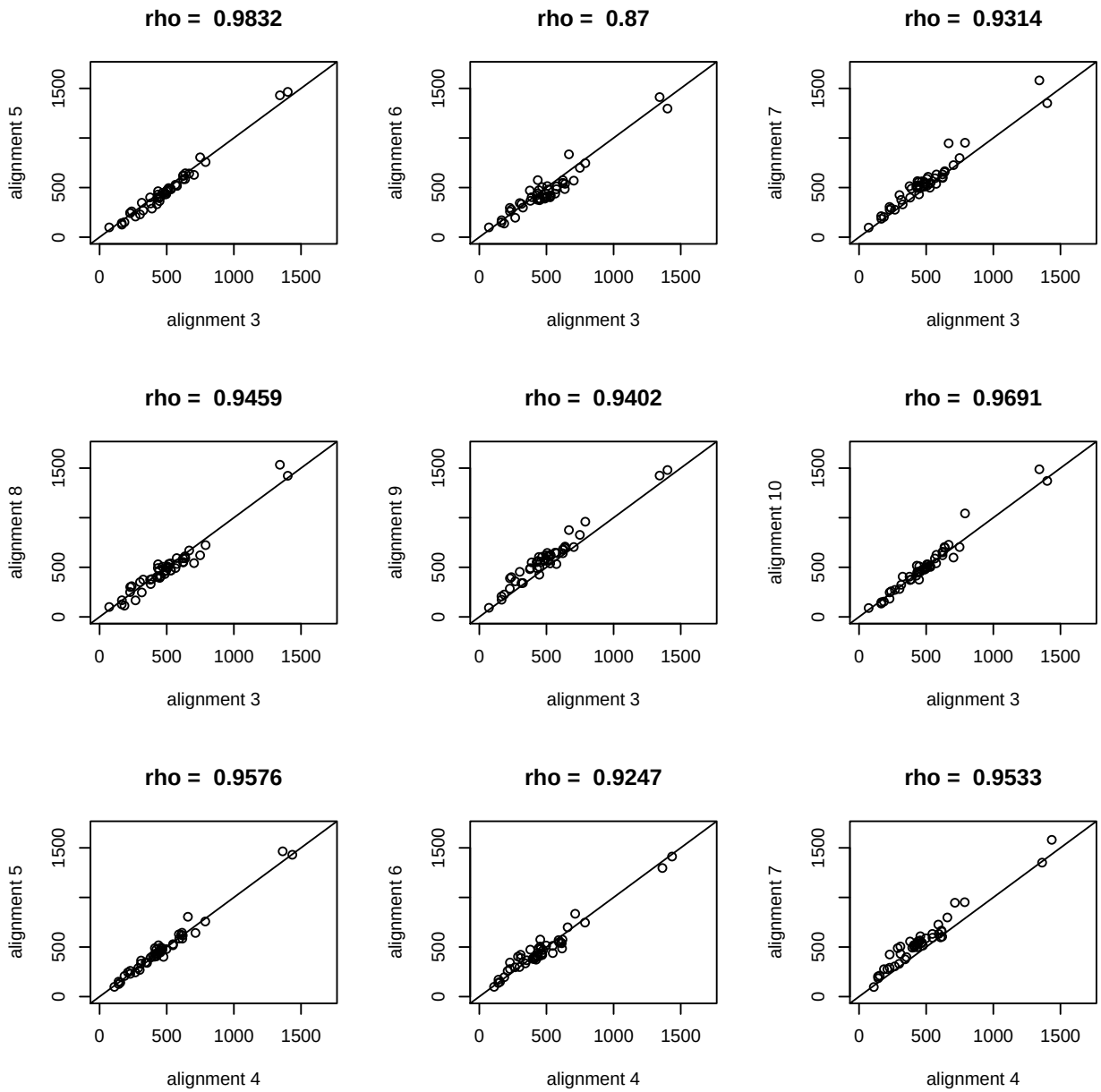


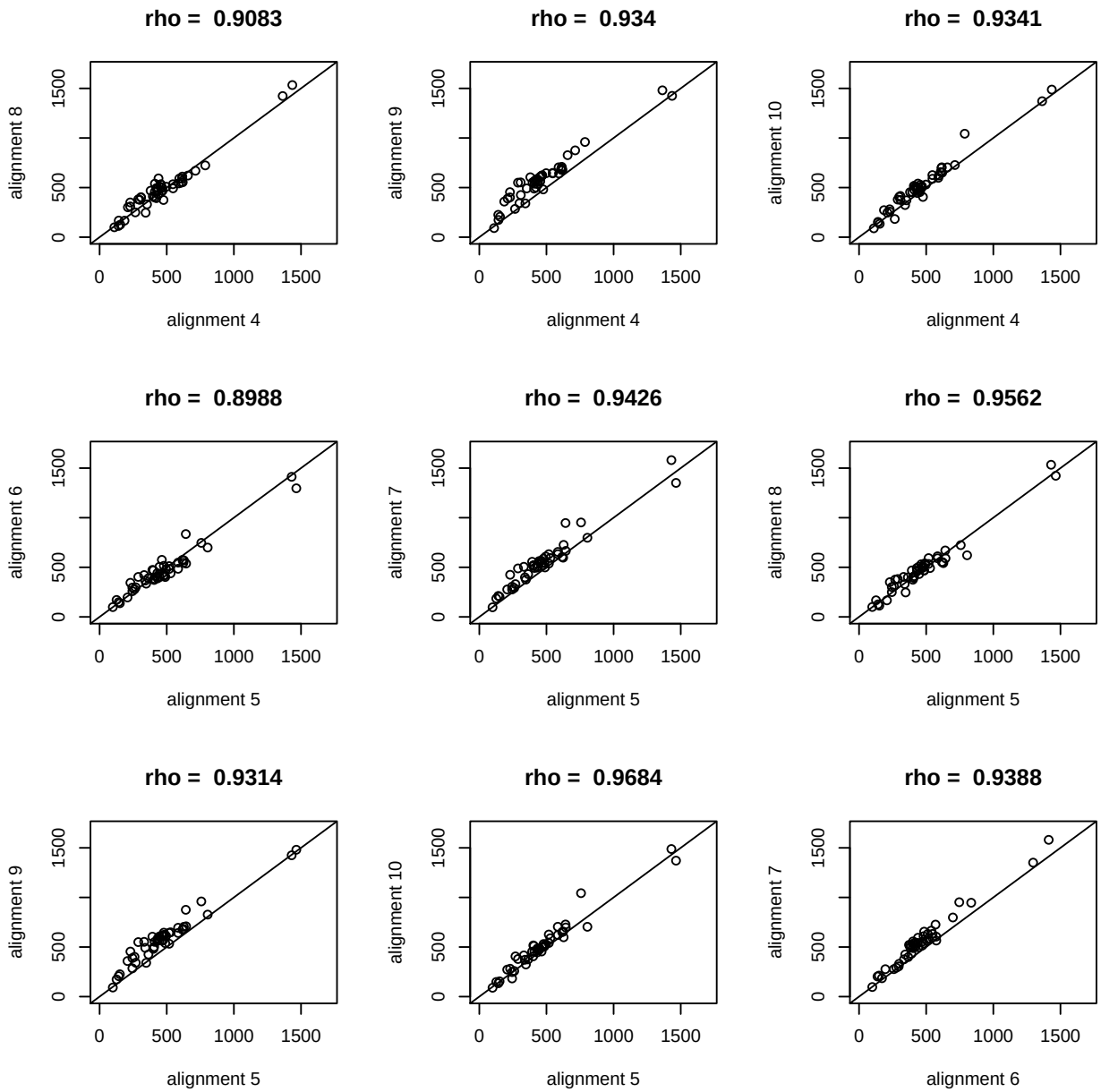
Supplementary file 6

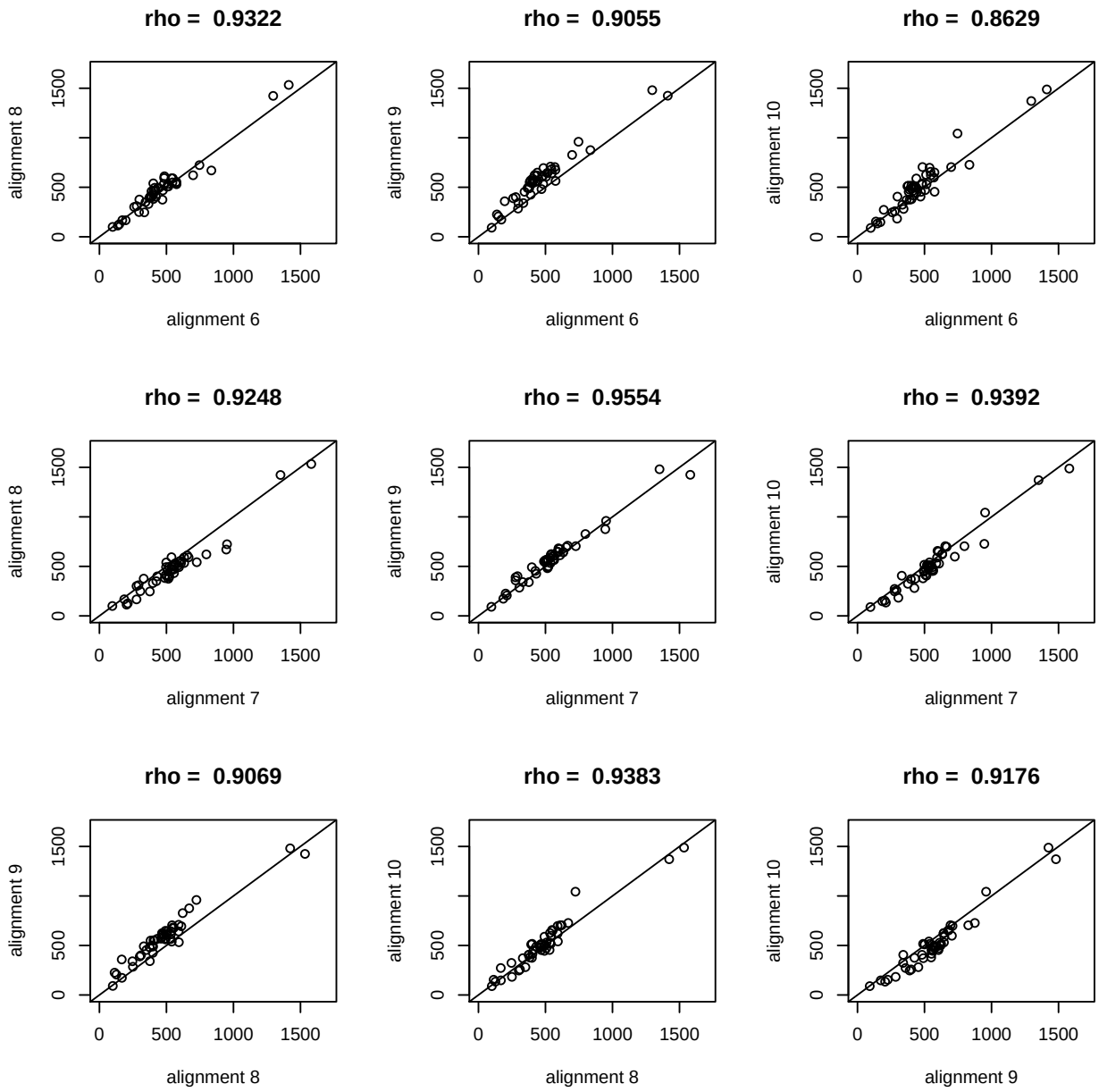
Estimates of reconstructed ancestral age of first female sexual maturity for a given branch are highly correlated between Coevol chains run on different concatenated sequence alignments. Plots show pairwise correlations, with the x and y axes representing age of 1st maturity for the labelled alignment and rho denoting Spearman's rank correlation coefficient. These results indicate that the alignments were sufficiently long to give reproducible results.


rho = 0.9531
rho = 0.9407
rho = 0.9549
rho = 0.9529
rho = 0.9396
rho = 0.9391
rho = 0.9459
rho = 0.9578
rho = 0.9231
alignment 1
alignment 2
alignment 3
alignment 4
alignment 5
alignment 6
alignment 7
alignment 8
alignment 9
alignment 10
0
500
1000
1500


rho = 0.9658
alignment 3
alignment 2
rho = 0.9588
alignment 4
alignment 2
rho = 0.9728
alignment 5
alignment 2
rho = 0.9036
alignment 6
alignment 2
rho = 0.9594
alignment 7
alignment 2
rho = 0.9409
alignment 8
alignment 2
rho = 0.9423
alignment 9
alignment 2
rho = 0.9598
alignment 10
alignment 2
rho = 0.93
alignment 4
alignment 3
0
500
1000
1500


rho = 0.9832
alignment 5
alignment 3
rho = 0.87
alignment 6
alignment 3
rho = 0.9314
alignment 7
alignment 3
rho = 0.9459
alignment 8
alignment 3
rho = 0.9402
alignment 9
alignment 3
rho = 0.9691
alignment 10
alignment 3
rho = 0.9576
alignment 5
alignment 4
rho = 0.9247
alignment 6
alignment 4
rho = 0.9533
alignment 7
alignment 4
0
500
1000
1500


rho = 0.9083
alignment 8
alignment 4
rho = 0.934
alignment 9
alignment 4
rho = 0.9341
alignment 10
alignment 4
rho = 0.8988
alignment 6
alignment 5
rho = 0.9426
alignment 7
alignment 5
rho = 0.9562
alignment 8
alignment 5
rho = 0.9314
alignment 9
alignment 5
rho = 0.9684
alignment 10
alignment 5
rho = 0.9388
alignment 7
alignment 6
0
500
1000
1500


rho = 0.9322
alignment 8
alignment 6
rho = 0.9055
alignment 9
alignment 6
rho = 0.8629
alignment 10
alignment 6
rho = 0.9248
alignment 8
alignment 7
rho = 0.9554
alignment 9
alignment 7
rho = 0.9392
alignment 10
alignment 7
rho = 0.9069
alignment 9
alignment 8
rho = 0.9383
alignment 10
alignment 8
rho = 0.9176
alignment 10
alignment 9
0
500
1000
1500

Supplementary file 7

Species 1	Species 2
Meleagris gallopavo	*Gallus gallus*
Merops nubicus	*Picoides pubescens*
Apaloderma vittatum	*Buceros rhinoceros*
Leptosomus discolor	*Colius striatus*
Cathartes aura	*Haliaeetus albicilla*
Taeniopygia guttata	*Geospiza fortis*
Corvus brachyrhynchos	*Manacus vitellinus*
Melopsittacus undulatus	*Nestor notabilis*
Falco peregrinus	*Cariama cristata*
Egretta garzetta	*Pelecanus crispus*
Nipponia nippon	*Phalacrocorax carbo*
Aptenodytes forsteri	*Pygoscelis adeliae*
Gavia stellata	*Fulmarus glacialis*
Eurypyga helias	*Phaethon lepturus*
Balearica regulorum	*Charadrius vociferus*
Calypte anna	*Chaetura pelagica*
Chlamydotis macqueenii	*Tauraco erythrolophus*
Mesitornis unicolor	*Pterocles gutturalis*
Phoenicopterus ruber	*Podiceps cristatus*