

## Introduction

In 1986 John Pettigrew and his team at The University of Queensland published a paper proposing that megabats (megachiroptera) were in fact more closely related to primates than they were to microbats (microchiroptera), and that the state of being a bat evolved twice (Pettigrew 1986). There are several physiological similarities between megabats and primates, including several visual pathways, behavioral characteristics, motor pathways and hippocampal structures (Pettigrew 1986). These pathways are not seen in microbats, nor are they seen in any other mammals but primates. Yet all the traditional trees grouped megachiroptera and microchiroptera together, including those based on DNA data. If megabats really were descendants of primates, why do they group with microbats in traditional phylogenetic trees?

Pettigrew proposed the idea – known as the ‘flying DNA’ hypothesis – that bats went on a DNA diet; they got rid of the heavier guanine and cytosine in their DNA sequences in favor of lighter adenine and thymine (Pettigrew 1995). He hypothesized that because flight is so metabolically costly, animals whose DNA consists of more A’s and T’s (the lighter base pairs) will have an advantage over those with more G’s and C’s (the heavier, more stable base pairs). Both mega and micro bats would exhibit a similar trend towards this AT bias, and thus their genomes would be pulled together in the tree not by actual similarity, but by the advantages of having more A’s and T’s (Pettigrew 1995). This was why they were always grouped together on the phylogenetic tree.

In his paper, Pettigrew looked at the difference in base composition between several different types of DNA: mitochondrial and nuclear, poikilotherm and homeotherm, small mammals and large mammals and finally megabats, microbats and primates. As the organism requires more energy to survive and the cells become more energetic Pettigrew saw an increase in the proportion of adenine and thymine. A later paper however, published by Ronald A. Van Den Bussche from Texas Tech University refuted Pettigrew's findings (Van Den Bussche 1998). They conducted their own analyses and found no evidence for the “flying DNA” hypothesis.

I wanted to look at this AT bias, and see if, in a small scale study, this idea would hold up not just in bats but in other flighted organisms. If the advantage of having more A's and T's is substantial enough to get primates into the air, it is possible that the same such transition happened within other flying groups like birds and insects.

## Methods

### *Bats*

First I wanted to address the organisms at the source of the “flying DNA” hypothesis: Pettigrew's bats. In order to make comparisons based on phylogeny, I identified up a few hypotheses. (1) Megabats and microbats would both have elevated levels of the AT pair compared to GC. (2) The closest relatives to both megabats (primates in Pettigrew's hypothesis) and microbats (possible ant-eaters) would have lower levels of the AT pair. In order to make the comparison, I selected a mitochondrial gene because Pettigrew hypothesized that the AT elevation would be higher in a more active mitochondrial sequence than a nuclear one.

Recent studies indicate that within placental mammals, bats may actually be more closely related with the order Carnivora, comprised of dogs, skunks, weasals, seals, bears, cats, raccoons etc (Van Den Bussche 1998). Based on this I looked at four groups to make the AT comparison: megachiroptera, microchiroptera, primates and carnivora. Figure 1 shows my hypothetical tree, based on the conventional family trees (not taking AT bias into account), as well as the tree Pettigrew predicts in his paper.

In order to analyze all four of these groups I selected specific sets of species that lined up with both Pettigrew and Van Den Bussche's papers. To represent megabats I chose the genus *Pteropus*, and to represent microbats I chose the genus *Myotis*. Primates and Carnivora are represented by a wide selection of species within each category. Table 1 shows the representatives of each group, as well as their DNA base pair composition.

To build the tree I compiled the 12S FASTA data from Genbank and aligned it in Mesquite with Muscle. I compiled the base frequencies in PAUP. With Mr.ModelBlock I found that the best model was the GTR+I+G model, and I used that to conduct a Mr. Bayes analysis of 100,000 generations. The resulting tree is shown in Figure 2, and is the result of a burnin value of 25%. The node labels are the posterior probability values from this analysis.

### *Birds*

After compiling data on the bats and their close relatives, I wanted to investigate whether the AT bias was common amongst all flying organisms. It is possible that, if having an AT bias is beneficial for flying animals, that birds and their non-flying ancestors could show a similar trend. To keep the analysis consistent I used 12S for the bird groups as well.

To assess this I chose to compare ratites and their close flying relatives, Anseriformes (geese) to non-flying ancestors like crocodiles. I pulled sequences for species off Genbank based on their availability (Table 1) and used PAUP to calculate the base pair frequency in the sequence for each taxon. The current relationship between neoaves, crocodiles and ratites is relatively stable (Figure 3). One would expect that geese, who often fly long distances in migration patterns would have a higher AT content than non-flying ratites and crocodiles.

To build the bird tree I followed the same procedure as with the bats, compiled 12S FASTA data from Genbank, aligned in Mesquite with Muscle and analyzed with Mr. Bayes under a GTR+I+G model (found using Mr.ModelTest). The tree (Figure 4) had a burnin value of 30% and the node labels are Mr. Bayes values after a run of 100,000 generations.

### *Insects*

Both birds and bats are relatively large bodied organisms, so I wanted to look at a smaller set of animals as they would be more strongly affected by small weight changes implied by the flying DNA hypothesis.

I took representatives from un-winged insect in subclasses Thysanura and Archaeognatha as well as flighted insects from the subclass Pterygota (Table 1). The current relationship between these subclasses is well founded (Figure 5). If the AT bias were to hold true here, we would expect that Pterygota would have a higher proportion of AT than Thysanura and Archaeognatha.

To build the tree I compiled 12S data from Genbank into Mesquite, aligned with Muscle and used an analysis of 100,000 generations in Mr.Bayes (under at GTR+I+G model found with Mr.ModelTest). The resulting tree (Figure 6) had a burnin value of 10% and shows the Bayes values at the nodes.

## **Results**

### *Bats*

My tree (Figure 2) corresponds neither with the currently accepted tree, nor with Pettigrew's flying DNA hypothesis. Megabats and microbats were indeed separated, however megabats were more closely related with Carnivores than Primates. When you compare the tree with the composition of the genomes (Figure 7) it is clear that, in fact, all organisms show a bias towards A and T.

The support values on the Bayes tree are relatively high for many of the nodes, and highly support separate megachiroptera and microchiroptera clades (both have posterior probabilities of .99). Perhaps more interesting is that in the tree for the 12S, megachiroptera were more closely related to carnivores than they were to microchiroptera, however the node that separates the megachiroptera/carnivore clade and the microchiroptera clade had a relatively low posterior probability of 0.6. The separation between both these clades and the primates is better supported (posterior probability of 0.78).





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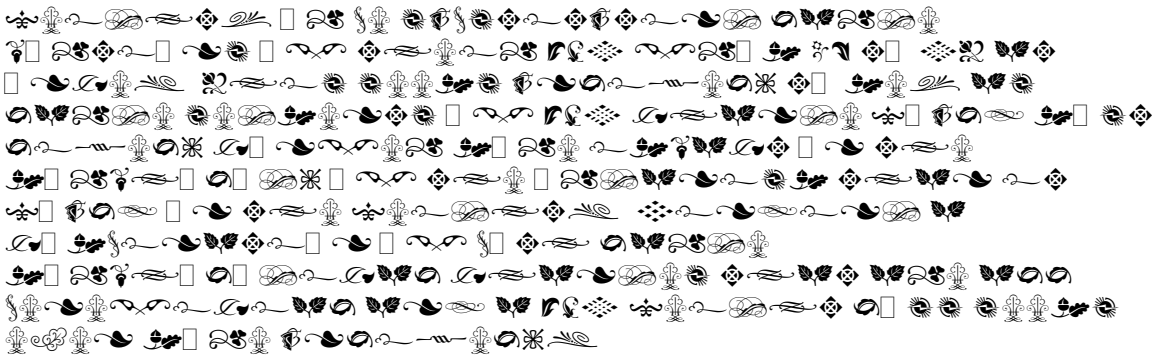
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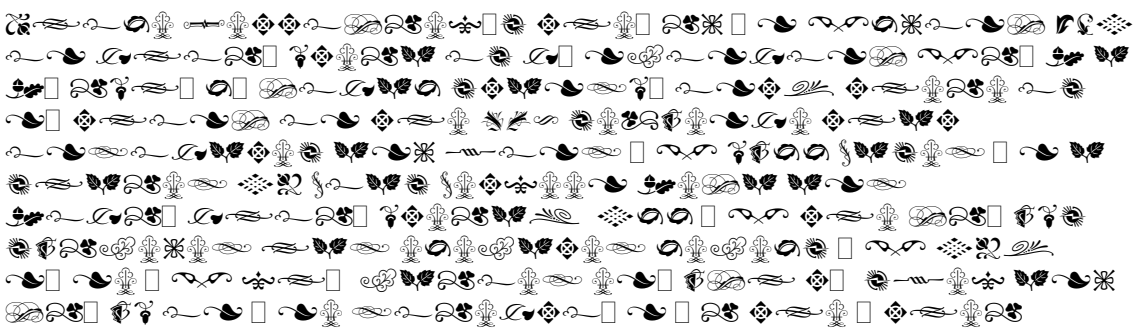
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## Discussion

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The bat tree (Figure 7) based only on AT/GC composition, actually mimics Pettigrew’s proposed tree, the one that was supposed to remedy the supposed AT bias.

*Birds*

The birds here are, perhaps, not a good comparison to bats in the sense that they have followed a very different evolutionary path. Where Pettigrew hypothesizes that bats evolved flight from primates, the ratites had flight and then lost it. If his theory is correct, ratites would have, at one point, had the AT bias and could perhaps still have it. Because they are now land dwelling, the benefit of an AT skewed genome is lost, however this does not mean that the AT bias itself was lost.

That said, the fact that ratites had the highest level of AT doesn’t seem to fit into Pettigrew’s hypothesis, or the evolutionary model here. Even if ratites at one point had just as much AT as geese or other flying birds, there is no reason that they should continue substituting AT for GC once they lost flight. There would be no advantage to it. The elevated AT in ratites only fits into the flying DNA hypothesis if ratites had this elevated level long ago, when they could fly, and have yet to lose it.

It is certainly possible that the flying ancestors of ratites had higher AT levels than modern geese, however the levels shown here do not indicate that they have biased the tree in any way. The tree based on AT/GC alone for birds (Figure 7), and it would have produced a different tree than the one found in this study.

## *Insects*

As discussed earlier, one way the evolution of an AT bias might be feasible is if the organisms were effected by extremely small changes in weight. This is why I wanted to look at insects, which are a more likely candidate to benefit from miniscule weight losses.

The insect tree is interesting because it does exhibit some evidence for what an AT bias can do to a tree. The tree based only on AT composition (Figure 7) is the same tree that we found in this study (Figure 5), and not the one that is accepted by current theories. It is possible that the tree shown here was effected by the AT pattern between the three groups. This is evidence that if there were a strong AT bias among groups it would indeed affect the tree (although this is not the troublesome part of Pettigrew's hypothesis).

Based on the small difference between species within the three groups evaluated here in AT bias, I don't believe that the 12S sequence here shows any kind of evidence for the AT bias being a factor that could skew the phylogenetic tree. That said, this is just one gene and could be a poor representation of the actual base composition of the overall genome. More evaluation should be done on the complete genome – both mitochondrial and nuclear.

Evolutionary models should also be taken into account when tackling this issue of bias in the DNA. The comparison between birds and bats here, for example, brings up the issue of making comparisons based on different evolutionary pathways. It is difficult to tease apart what might be an AT bias and what might be different evolutionary pathways at work. We cannot assume that the AT bias found in bats has happened for the same reason as an AT bias found in insects. Perhaps having AT pairs confers some other advantage to organisms – which seems likely due to the occurrence of such a bias in every organism surveyed in this study.

## **Works Cited**

- Murphy, William J., Thomas H. Pringle, and Tess A. Crider. "Using genomic data to unravel the root of the placental mammal phylogeny." *Genome Research* (2007). *Genome Research*. Cold Spring Harbor Laboratory Press, 28 Nov. 2007. Web. <genome.org>.
- Pettigrew, John D. "Flying Primates: crashed, or crashed through?" *Zoological Symposium* 67 (1995): 3-26. Print.
- Pettigrew, John D. "Flying Primates? Megabats Have the Advanced Pathway from Eye to Midbrain." *Science* 231 (1986): 1304-306. Print.





Figure 3: Currently accepted relationship between Ratites (Palaeognathae) and Anseriformes (Neoaves) produced by tolweb.org.

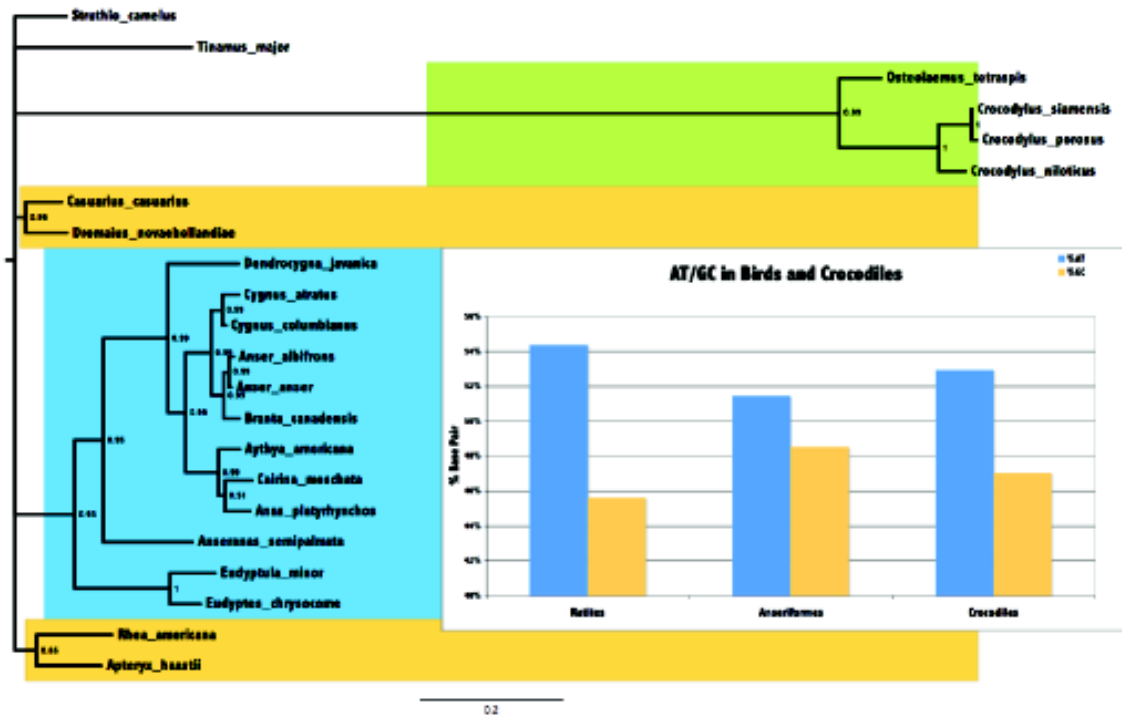


Figure 4: Tree of Anseriformes, Ratites and Crocodyla based on a Bayes analysis of 100,000 generations under a GTR+I+G model. Graph shows the %AT/GC composition of Ratites, Anseriformes and Crocodyla.

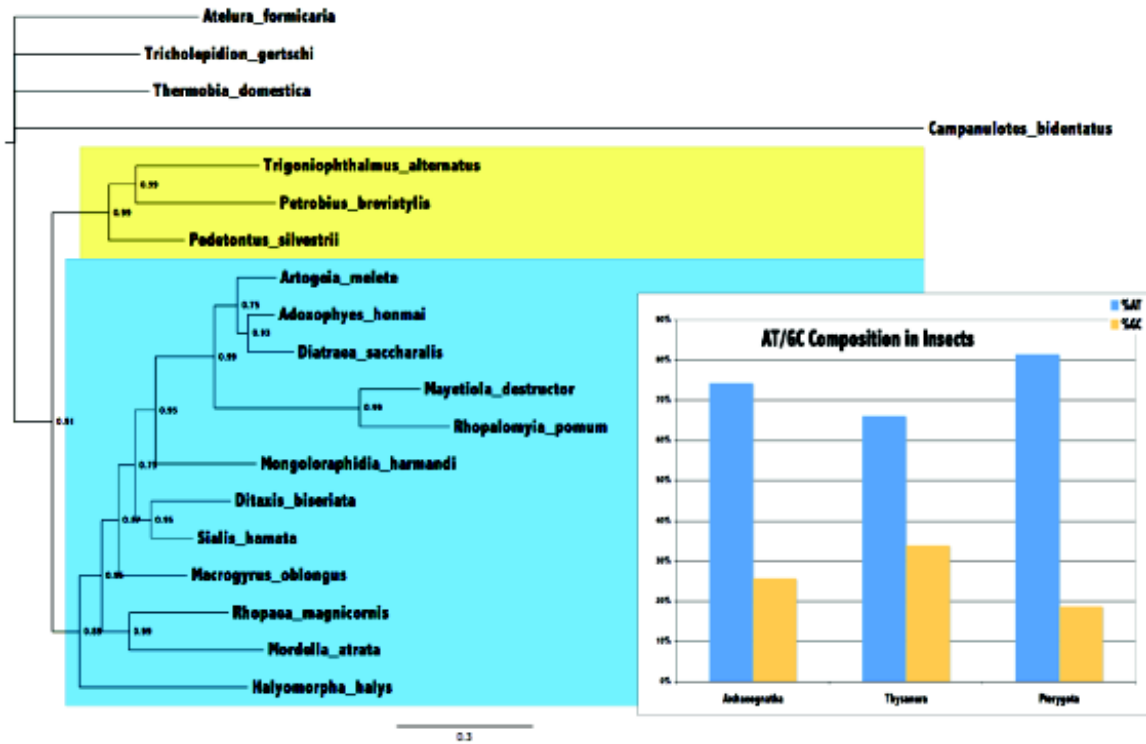


Figure 6: Insect tree based on a Bayes analysis of 100,000 generation under a GTG+I+G model. Graph shows the %AT/GC composition of the three Archaeognatha, Thysanura, Pterygota.

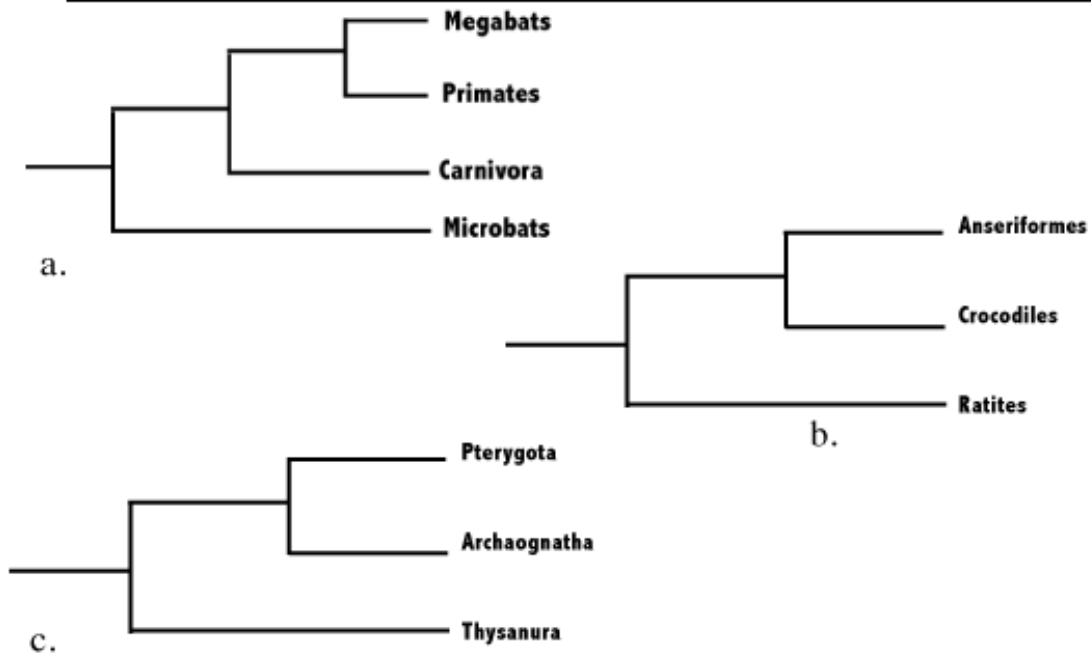


Figure 7: Trees based on AT/GC composition alone.

	%AT	%GC
<b>Ratites</b>	54.24%	45.76%

Struthio camelus	52.44%	47.56%
Rhea americana	53.88%	46.12%
Casuarius casuarius	55.73%	44.27%
Apteryx haastii	55.08%	44.92%
Dromaius novaehollandiae	54.90%	45.10%
Tinamous major		
<b>Anseriformes</b>	52.25%	47.76%
Dendrocygna javanica	51.47%	48.53%
Cygnus atratus	51.82%	48.18%
Anser albifrons	51.12%	48.88%
Aythya americana	52.47%	47.53%
Cygnus columbianus	51.92%	48.08%
Anser anser	50.40%	49.60%
Cairina moschata	50.56%	49.44%
Anas platyrhynchos	51.68%	48.33%
Branta canadensis	50.87%	49.13%
Anseranas semipalmata	51.49%	48.51%
Eudiptula minor	51.53%	48.47%
<b>Crocodyles</b>		
Osteolaemus tetrapis	54.63%	45.36%
Crocodylus siamensis	52.18%	47.82%
Crocodylus porosus	52.34%	47.66%
Crocodylus niloticus	52.69%	47.31%
<b>Megabats</b>		
Pteropus dasymallus	57.93%	42.07%
Pteropus aldabrensis	57.62%	42.38%
Pteropus scapulatus	57.85%	42.15%
Pteropus seychellensis	57.84%	42.16%
Pteropus vampyrus	57.16%	42.84%
Pteropus voeltzkowi	57.13%	42.87%
<b>Microbats</b>		
Myotis adversus	60.25%	39.75%
Myotis albescens	61.14%	38.86%
Myotis austroriparius	60.75%	39.25%
Myotis californi	60.87%	39.13%
Myotis capaccinii	61.34%	39.13%
Myotis ciliolabrum	61.08%	38.92%
Myotis daubentoni	61.30%	38.70%
Myotis dominicensis	61.24%	38.76%
Myotis elegans	60.19%	39.81%
Myotis fortidens	61.08%	38.92%
Myotis keaysi	60.93%	39.07%

Myotis muricola	61.63%	38.37%
Myotis myotis	61.08%	38.92%
Myotis nigricans	65.58%	37.42%
Myotis ridleyi	62.10%	37.90%
Myotis ruber	60.60%	39.40%
Myotis serptentrionalis	61.08%	38.92%
Myotis siligorensis	60.62%	39.38%
Myotis thysanodes	61.83%	38.68%
Myotis vellifer	60.93%	39.07%
Myotis volans	61.12%	38.88%
Myotis welwitschii	62.37%	37.63%
Myotis yumanensis	61.24%	38.76%
<b>Tamandua</b>		
Tamandua tetradactyla	56.58%	43.42%
<b>Primates</b>		
Homo sapiens	54.19%	45.81%
Lemur catta	59.60%	40.40%
Cynocephalus variata	56.58%	43.42%
<b>Carnivora</b>		
Canis lupus	60.48%	46.23%
Cuon alpinus	59.27%	46.39%
Vulpes Vulpes	59.25%	45.77%
Uncia uncia	58.13%	46.25%
Spilogale putorius	60.70%	44.97%
Odobenus rosmarus	59.96%	45.88%
Felis catus	58.44%	45.94%
Halichoerus grypus	58.33%	45.52%
Ursus thibetanus	58.63%	45.71%
Acrodon simus	56.91%	45.86%
Ursus spelaeus	58.67%	45.79%
Ailurus fulgens	61.10%	46.06%
Meles meles	60.90%	45.15%
Gulo gulo	58.79%	45.16%
Martes melampus	58.86%	45.11%
Enhydra lutris	59.81%	45.48%
Neofelis nebulosa	58.35%	45.83%
<b>Table 1: AT and GC composition by species</b>		