Supplementary Discussion of Results

Evolution of G/A gradients

In the hierarchical clustering (Figure 2a and Table 3), clusters that were not rejected at the 0.05% significance level (likely clusters with $\Delta \ln L$ less than 3.0) included one large set of species (Group 10) and five species pairs (Groups 5-9) that were sometimes but not always closely related to one another. At moderately large cost (somewhat unlikely clusters, but $\Delta \ln L$ still less than 10.0), each of these groups merged with another or with the two previously unclustered species to form four new groups (Groups 11-14). The next two mergers (Group 15, the deep-branching primates and outgroups, and Group 16, the great apes and Old World monkeys) were more incredible ($45 > \Delta \ln L > 60$), while all primates and outgroups could only be merged together as one group at an extremely unbelievable cost of $\Delta \ln L = 497$.

One difficulty in interpreting these results is that the order of clustering can strongly affect whether particular species join together early or late in the hierarchical clustering process. For example, in pairwise comparisons, humans could easily have joined together with the gorilla, chimpanzee, and pygmy chimpanzee with only a small decrease in likelihood ($\Delta \ln L \sim 3$). They joined most easily with the gibbon, however, thus being led away from the other great apes. This order of clustering meant that the baboon/macaque cluster was subsequently slightly more likely to join the gorilla/chimp/pygmy chimp cluster than was the human/gibbon cluster. The human/gibbon cluster was then more likely to join the orangutans than this combined cluster, and all the great apes and Old World monkeys joined together at the rather high cost of 59.6 log likelihood units (Supplementary Data, Table B). Other interesting points are that the intercept tended to matter more in clustering than the slope, and as expected, clusters were more easily joined when a slightly smaller intercept was balanced with a slightly bigger slope.

Mixture model analysis offers an alternative means of assessing similarity among responses to the gradient that is not order dependent. In such analyses, all species were evaluated simultaneously (the outgroups were excluded), and the best set of models was determined (Supplementary Data, Table C). Although individual species were not deterministically linked to a specific model, the posterior probability that data from a particular species was generated by each model can be calculated (Equation 5). For six models or fewer, the posterior probability for each species was approximately one for one of the models and approximately zero for the others, although in ten replicates there was some variance in the posterior for the five and six model cases (data not shown). Clustering is obviously related to the results from the hierarchical analysis, but due to the non-hierarchical nature of the mixture analysis, switches in alliances among groups can occur for different numbers of clusters. For example, with three models (Figure 2b), humans clustered with the orangutans and gibbons (Group X), as before, while the other great apes clustered with the Old World monkeys (Group Y), and the remaining primates all clustered together (Group Z). With five models, the deeper primates split into two groups (Groups W and V), as did the great ape/Old World monkey mixed group (Groups T and U). In the latter case, two of the Old World monkeys split off, but the baboons remained in a cluster with the hominids, which included humans, as expected based on phylogenetic relatedness. In Figure 3 are shown the posterior probabilities that each species belongs to each of these models; although the ML results discussed above definitively place the different species with particular models, the posterior allegiances are often shared between models when they are adjacent to one another. If these clusters are mapped onto a phylogenetic tree (Figure 4), it is clear that the baboons, and to some extent all of the Old World monkeys, have converged to a similar response curve as the hominoids.

Evolution of C/T and Y/R gradients

Although the C/T ratio did not show a clear slope in our earlier study (Faith and Pollock 2003), we performed individual and hierarchical analyses on the C/T ratio response to single-strandedness to determine if there was any variation in the level of asymmetry or the existence of a slope among the primates (Supplementary Data, Tables D and E). We also performed these analyses on the Y/R ratio at 4x redundant 3rd codon positions to see if there was detectable variation in slopes and intercepts for transversions (Supplementary Data, Tables F and G). In the C/T analysis, there are three discrete clusters that that were not rejected at the 0.05% significance level (Table 4), but required more substantial penalties ($\Delta \ln L$ were 8.5 or more) to merge (Figure 2c). The largest group (Group 13, nine species) has a strong bias against C and a slightly negative but not significant slope, indicating increasing bias against C with increasing single-strandedness. Another group (Group 14) is an odd phylogenetic assortment of primates and the two non-primate outgroups, and has substantially less bias against C than Group 13 The phylogenetic separation of these species indicates that there may be a recurrent mechanism by which bias against C may be reduced, presumably by increasing protection against or repair of the causative mutation. Results with the C/T ratio are very tentative because of the non-linear response, and indeed, studies currently underway indicate that there is considerable complexity in the evolution of this response curve.

The Y/R ratio analysis of individual genomes also proved interesting, in that *Tupaia* was the only organism with a significant slope (Figure 2d, Table 4, Supplementary Data, Table F). *Tupaia* had an even ratio of pyrimidines to purines at zero $DssH$, but had a positively increasing bias toward pyrimidines with increasing $DssH$, and did not group with the three likely cluster (Groups 6, 12, and 14, with four, five, and three species respectively). Creation of the next two clusters (Groups 15 and 16) required large $\Delta \ln L$ penalties (11.4 – 11.8; Supplementary Data, Table G), but there was an extremely large $\Delta \ln L$ penalty (61.5) to merge these last two cluster. While group 15 was biased towards purines with a nearly constant Y/R ratio of 0.867, Group 16 began with an equal ratio of pyrimidines to purines and had an increasing bias towards pyrimidines with increasing $DssH$. The generally flat slopes in the primates provided little evidence for excess transversion mutations in response to single-strandedness, although the significant slope in *Tupaia* (and the significant slope for the combined members of Group 16) is preliminary evidence that such a response can exist in some organisms (and is perhaps usually controlled by efficient repair mechanisms). Interestingly, *Tarsius* did not group with the strepsirrhines and outgroups based on the Y/R ratio, while the deepest-branching New World monkey, *Cebus*, did, although the differences between the tarsier and *Lemur* were not large (Supplementary Data, Tables F and G).