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Response to Comment on “Chromosomal Speciation and Molecular Divergence—Accelerated Evolution in Rearranged Chromosomes”

By clever use of outgroups, Lu *et al.* (1) tackle some of the questions raised by Navarro and Barton (2). Beyond confirming the previous result that rapidly evolving genes tend to be associated with chromosomes that have been rearranged between humans and chimpanzees (2), Lu *et al.* make the remarkable observation that the association is much older than the human-chimpanzee split. We fully agree with their interpretation that, because the outgroups split from the human-chimpanzee lineage between 12 and 35 million years ago, the high K_A/K_S ratios in rearranged chromosomes cannot be attributed primarily to the particular rearrangements separating humans and chimpanzees (which only occurred in the last 6 to 7 million years). Nevertheless, we believe that the existence of these two correlations—clusters of positively selected genes that in turn show a strong association with recently rearranged chromosomes—needs an explanation and that this explanation might be connected with speciation.

First, let us consider whether any differences in rates of protein evolution between rearranged and colinear chromosomes could be associated with the recent rearrangements that separated humans and chimpanzees. When computing K_A/K_S averages, we ignored genes with $K_S = 0$ (2). In contrast, Lu *et al.* computed the K_A/K_S values of these genes by setting $K_S = 0.002$. This biases toward much higher K_A/K_S ratios in smaller genes. Also, small changes in these K_S values imply large changes in K_A/K_S ratios (e.g., using 0.001 would double some already large K_A/K_S values; most of the largest K_A/K_S values in the data set arise in this way). Introducing this arbitrary lower limit to K_S has a greater impact on the K_A/K_S ratios of the human-chimpanzee comparison than on the human-outgroup comparison (because the latter has fewer $K_S = 0$ values). Also, in the data used by Lu *et al.*, K_A/K_S values are more affected in colinear than in rearranged chromosomes. If we ignore genes with $K_S = 0$ in that data set, the K_A/K_S ratio for rearranged and colinear chromosomes

(R/C) for the human-chimpanzee comparison is larger than the R/C ratio for human-outgroup comparison (1.65 versus 1.42). Furthermore, rearranged chromosomes tend to have a larger number of genes with $K_A/K_S > 1$ than colinear chromosomes. However, this proportion is 2.04 for the human-chimpanzee comparison and only 1.37 when comparing humans with outgroups. Although these are not large differences, they hint at an acceleration of protein evolution in association with recent rearrangements.

To further examine this possibility, it is convenient to estimate the K_A/K_S ratios of the branches leading to humans, chimpanzees, and outgroups. Because this cannot be achieved by means of pairwise comparisons, we reanalyzed the Lu *et al.* data set (1) with the maximum likelihood method in PAML (3). The R/C ratio was 1 in all branches, reinforcing their idea of an old clustering of positively selected genes. Interestingly, the branch leading to humans always showed higher R/C ratios than that leading to outgroups (1.76 versus 1.58 using unrooted trees, and 2.05 versus 1.15 using rooted trees). Again, this suggests a recent increment in the association between rearrangements and high K_A/K_S ratios.

Let us now consider the critical question of what could cause an association between rearrangements and rapidly evolving genes. There are a number of potential explanations, some of which we raised previously (2), and several of which are unrelated to speciation. For example, the establishment of rearrangements may change the expression of associated genes, which may trigger amino-acid changes; or rearrangements may be established more readily if they hitchhike with positively selected variants, which may occur more frequently near clusters of rapidly evolving genes. Other potential explanations, including our model (4), suggest that regions with clusters of positively selected genes would be more likely to take part in speciation.

All of these possibilities generate interesting interpretations and suggest new analyses. However, there is a simpler and more intriguing factor to take into account. With-

in primates, some chromosomes have undergone intense rearrangement, whereas some syntenic groups have been conserved (5, 6). Moreover, breakpoints for rearrangements are highly conserved in primates (7, 8). This suggests that the genomic distribution of rearrangements is not random, but rather that the same regions have been rearranged over and over in different branches of the primate lineage. For instance, regions that have been rearranged between humans and chimpanzees (computed as rearrangements per chromosome per Mb) tend to be rearranged elsewhere in the lineage of the great apes (Spearman's correlation, $P < 0.05$, one-tailed). Whatever the reason for the association between high K_A/K_S ratios and rearrangements, it is not surprising that the genes and regions that show such association when comparing humans and chimpanzees should be involved in similar patterns when comparing humans and outgroups. These regions have been rearranged in the lineages that led to outgroups and, of course, they may have been implicated in speciation.

Finally, an important caveat must be raised. Hellmann *et al.* (9) analyzed human and chimpanzee divergence by comparing to their human orthologs more than 1200 random, highly-expressed chimpanzee expressed sequence tags (ESTs). They obtained an average K_A/K_S ratio of 0.22, almost threefold smaller than any previous result based on GenBank sequences. This opens the possibility of a bias in GenBank data that would affect both our results (2) and those of Lu *et al.* (1). In our view, which fully agrees with the final statement of Lu *et al.*, evidence for or against parapatric models of speciation is indeed elusive. Only the study of the full chimpanzee and human genomes, combined with those of outgroups, will settle these matters. We hope the necessary information will be available soon.

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TECHNICAL COMMENT

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