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Large Punctuational Contribution of Speciation to Evolutionary Divergence at the Molecular Level

Mark Pagel,* Chris Venditti, Andrew Meade

A long-standing debate in evolutionary biology concerns whether species diverge gradually through time or by punctuational episodes at the time of speciation. We found that approximately 22% of substitutional changes at the DNA level can be attributed to punctuational evolution, and the remainder accumulates from background gradual divergence. Punctuational effects occur at more than twice the rate in plants and fungi than in animals, but the proportion of total divergence attributable to punctuational change does not vary among these groups. Punctuational changes cause departures from a clock-like tempo of evolution, suggesting that they should be accounted for in deriving dates from phylogenies. Punctuational episodes of evolution may play a larger role in promoting evolutionary divergence than has previously been appreciated.

The theory of punctuated equilibrium as a description of evolution suggests that evolutionary divergence among species is characterized by long periods of stability or stasis followed by short punctuational bursts of evolution associated with speciation. Despite years of work on punctuational change, the theory remains contentious (1–9), with little or no consensus as to the contribution of punctuational changes to evolutionary divergence. The importance of the theory lies in the challenge it poses for classical accounts of how species diverge.

Punctuational evolution has traditionally been studied in the fossil record. However, phylogenetic trees derived from gene-sequence data contain the signatures of past punctuational and gradual evolution and can be used to study their relative contributions to evolutionary divergence (10) (Fig. 1). The nodes of a phylogenetic tree record the number of net-speciation events (speciation-extinction) between the root of the tree and the extant species (Fig. 1, A and B). In phylogenies derived from gene-sequence data, the lengths of the branches of the tree record the expected evolutionary divergence between pairs of speciation events, measured in units of nucleotide substitutions. We denote the sum of the branch lengths between the root of the tree and a species as the path length and

write this path length as $x = n\beta + g$, where n is the number of nodes along a path, β is the punctuational contribution of speciation to evolution at each node, and g is the gradual contribution to the path, this being the sum of the individual gradual effects in each branch along the path. Both parameters are measured in units of expected nucleotide substitutions per site in the gene-sequence alignment. Under a gradual model of evolution, there is no punctuational effect, $\beta = 0$, and there should be no relationship between x and n (Fig. 1, B and C). If, however, speciation events are associated with bursts of evolution, then $\beta > 0$, and path lengths from the root to the tips of

the tree will be correlated with the number of speciation events that occur along that path (Fig. 1, A and C).

We analyzed 122 gene-sequence alignments selected for including a well-characterized and narrow taxonomic range of species (11). This acts to control for background differences among species, such as generation times or adaptive radiation of some lineages, that might affect rates of evolution independently of a punctuational effect. For each data set, we derived a Bayesian sample of the posterior distribution of phylogenetic trees (11, 12). We then estimated β from the relationship between x and n in each tree in the posterior sample to account for phylogenetic uncertainty, using a statistical method (10, 13–15) that controls for the shared inheritance of branch lengths implied by the phylogeny (Fig. 1)

Using conservative statistical criteria (11), we found a significant relationship between nodes and path lengths (i.e., $\beta > 0$) in 57 [46.7 ± 4.5% (±SE)] of the 122 trees. We removed 22 of these data sets with $\beta > 0$ because they suffered from an artifact of phylogeny reconstruction known as the node-density effect, which can produce an apparent relationship between x and n (10, 11, 16–18). This left 35.0 ± 4.8% of the remaining 100 trees with significant effects of punctuational evolution (Fig. 2), rising to 55.8 ± 7.0% for trees above the median size of $n = 28$ taxa. The overall frequency of 35% is similar to that found in the subset of trees in which 50% of the known taxa have been

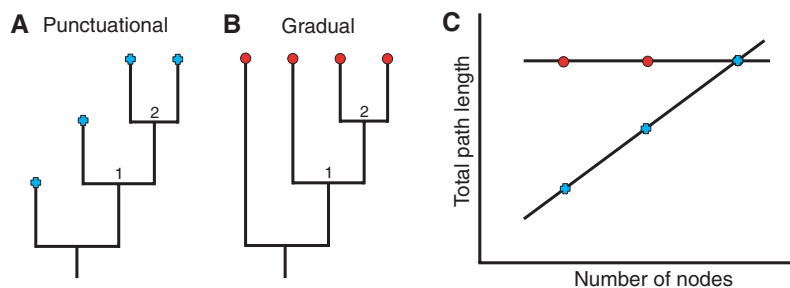


Fig. 1. Signatures of punctuational and gradual evolution on phylogenetic trees. (A) Punctuational evolution presumes a burst of evolution associated with each node of the tree. Path lengths, measured as the sum of branches along a path from the root to the tips of the tree, are proportional to the number of nodes along that path (C). Branches are assumed to be in units of nucleotide substitutions. (B) Gradual evolution presumes that change is independent of speciation events. Path lengths do not correlate with the number of nodes along a path (C). (C) Punctuational evolution predicts a positive relationship between path length and the number of nodes, whereas gradual evolution does not.

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sampled ($28.1 \pm 7.95\%$) and to that in data sets confined to a single genus ($32.0 \pm 6.60\%$). However, it is significantly more common in plants ($56.7 \pm 7.8\%$) and fungi ($71.4 \pm 16.0\%$) than in animals ($20.6 \pm 4.7\%$; $\chi^2 = 15.9$, $P < 0.0003$). This difference remains after controlling for the number of taxa in the phylogenetic trees ($P = 0.0063$) and after controlling for the amount of genetic diversity in the phylogeny [plants versus animals, $P = 0.003$; fungi versus animals, $P = 0.008$ (11)].

Our principal interest was to estimate the contribution of the punctuational effect at speciation to overall molecular diversity. The mean β is 0.0078 ± 0.0017 , ranging from 0.00024 to 0.057, but the absolute values depend on the evolutionary rate of the genes that were used to infer the phylogenetic tree. To control for this, we measured the proportion of the total amount of evolution on the tree attributable to punctuational effects. A bifurcating phylogenetic tree has $2(s - 1)$ branches where s is the number of

species. The length of a phylogenetic tree, T , is the sum of the lengths of these branches, here measured in units of nucleotide substitutions. The ratio $2(s - 1)\beta/T$, then, measures the proportion of the tree length attributable to punctuational effects.

If $\beta = 0$, there is no punctuational contribution and the ratio equals zero. If there are no gradual effects, then $2(s - 1)\beta = T$ and the ratio equals 1. We calculated the mean $2(s - 1)\beta/T$ value in the posterior sample of trees derived from each alignment. The mean value of these across the 35 data sets was 0.34 ± 0.08 (median = 0.30); where punctuational effects occurred in our sample, they contributed on average about 30% of the total molecular diversity. This is likely to be an overestimate, because in smaller trees only larger effects will tend to reach statistical significance. Figure 3 plots mean $2(s - 1)\beta/T$ against the number of taxa in the tree for the 35 data sets showing a punctuational effect. The fitted curve (11, 19) has an asymptote as the number of taxa approaches infinity, given by a parameter θ_1 , which here takes the value $\theta_1 = 0.22 \pm 0.036$. This estimate agrees well with the mean $2(s - 1)\beta/T$ of 0.23 ± 0.12 for trees with greater than the median number of taxa. Even though the frequency of punctuational effects varies among taxonomic groups (Fig. 2), the colored dots of Fig. 3 show that the contribution of punctuational effects to molecular diversity is roughly the same among plants, fungi, and animals.

Punctuational effects cause departures from a molecular clock-like tempo of evolution. By masquerading as gradual changes, punctuational effects will bias estimates of dates derived from molecular clocks that assume gradual evolution, making them occur too far in the past. Departures from the molecular clock are expected to vary with the size of β , as can be seen by studying the expected correlation, ρ , between path lengths and numbers of nodes:

$$\rho = \frac{\beta\sigma_n^2 + \sigma_{g,n}}{(\beta^2\sigma_n^2 + \sigma_g^2)^{1/2}(\sigma_n^2)^{1/2}}$$

Here, σ_n^2 is the variance in the number of nodes along the path lengths in the tree, and σ_g^2 is the variance in the gradual effects. The term $\sigma_{g,n}$ denotes the covariance between g , the gradual component of evolution in a path, and n , the number of nodes along the path, and is assumed to be zero. When punctuational effects do not operate, $\beta = 0$, all of the evolutionary change is gradual, and the expected correlation is zero (Fig. 1C). On the other hand, if all of the variation in path lengths arises from the punctuational effect, then $\sigma_g^2 = 0$, and the correlation simplifies to $\rho = \beta\sigma_n^2/\beta\sigma_n^2 = 1$.

Across the 35 data sets showing a punctuational effect, the average correlation be-

Fig. 2. Percentage of data sets with evidence for punctuational evolution. The Overall data set comprises the 100 trees free of the node-density artifact. 50% sampled refers to the subset of 42 trees in which 50% or more of the known taxa have been sampled. Plants, Animals, and Fungi are the data sets representing these taxonomic groups. Within Order, Within Family, and Within Genus isolate data sets of varying taxonomic range. Apart from differences among taxonomic groups, the rate of punctuational change is relatively stable among the various subsets. Error bars indicate the standard error of the mean.

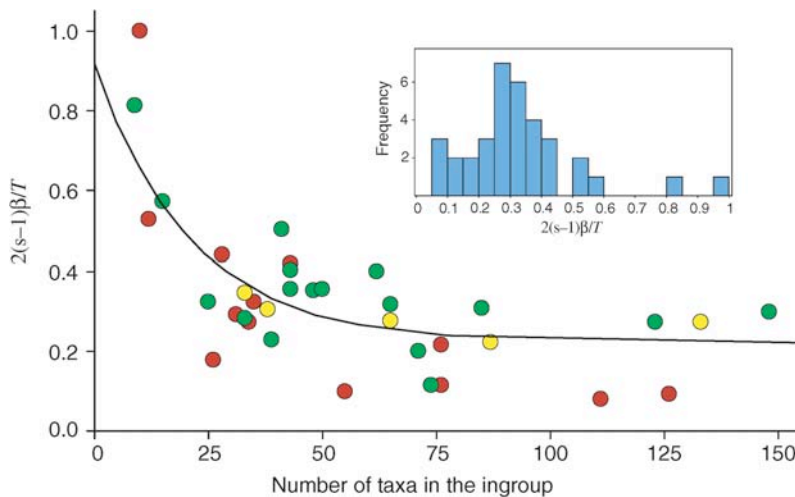
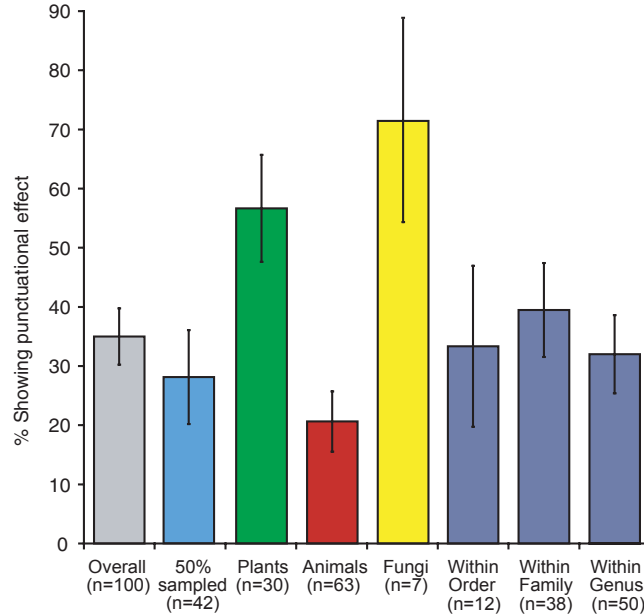


Fig. 3. Relationship between percentage of the tree length attributable to punctuational effects and tree size. Data points plot the mean $2(s - 1)\beta/T$ for each of the 35 phylogenies that show a significant punctuational effect against number of taxa in the tree. One tree, of 10 animal taxa, returned a $2(s - 1)\beta/T > 1.0$. Because this result, apart from sampling error, is impossible, we set this value to 1.0 in the graph and in Fig. 4. As the number of taxa approaches infinity, the curve has an asymptote of 0.22 ± 0.036 , indicating that punctuational effects contribute approximately 20% of the total molecular diversity on the tree. Although the rate at which punctuational effects occur is higher in plants and fungi than animals (Fig. 2) the mean contribution to total diversity is similar among groups: plants (green) = 0.31 ± 0.09 , animals (red) = 0.36 ± 0.04 , fungi (yellow) = 0.28 ± 0.10 . The inset displays a frequency histogram of all the $2(s - 1)\beta/T$ values.

tween path lengths and nodes ranges from 0.22 to 0.69 with a mean of $r = 0.42 \pm 0.019$; punctuational effects contributed between 4.8 and 48% (mean $r^2 = 0.42^2 = 0.18$) of the deviation from molecular clock-like behavior in these trees. The remaining variation in path lengths is attributable to variance in gradual effects that is independent of speciation. There is a close correspondence between these r^2 values and the size of the punctuational contribution, as measured by $2(s-1)\beta/T$. Trees with a larger punctuational component show stronger systematic departures from clock-like evolution (Fig. 4, $r = 0.79$, $P < 0.0001$). When phylogenetic trees derived from gene-sequence data are used to estimate divergence times, punctuational evolution should be tested for and statistically removed before inferring dates.

Punctuational effects appear to be widespread and common. Alternatively, we might be detecting lineages with intrinsically higher rates of evolution that speciate more as a result; this could produce apparent punctuational effects in the form of $\beta > 0$. We think this explanation is unlikely because it has proved difficult to find traits associated with rates of evolution, apart from generation time in a small number of cases (20), and these lineage effects would have to be widespread to explain our results. The narrow taxonomic ranges of most of our data sets also mean that the species will have similar morphologies and life histories. A related possibility is that rather than measuring a burst of evolution, we are detecting a generalized increase in the rate of evolution after speciation. This explanation depends on the elevated rate persisting through time and over many branches of the

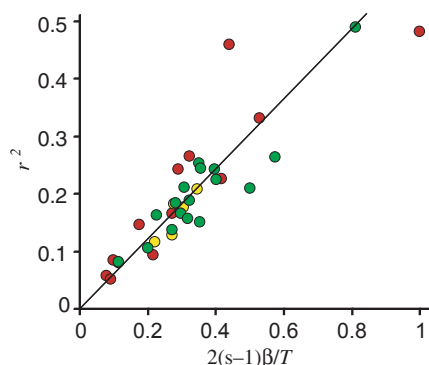


Fig. 4. Relationship of punctuational effects to divergence from the molecular clock. Plot of the correlation between path length and number of nodes (r^2) versus $2(s-1)\beta/T$ values. The r^2 values record the strength of the relationship between path lengths and numbers of nodes under a punctuational model (Fig. 1); r^2 is expected to be zero when evolution is gradual. The plot shows that when the punctuational contribution is large, as given by $2(s-1)\beta/T$, there is greater deviation from clock-like behavior.

tree. If the rate declined to background between speciation events but was assumed to be “recharged” by successive events of speciation, the phenomenon becomes indistinguishable from punctuational change. Elevated rates associated with lineages or speciation also fail to explain why we found such large differences among taxonomic groups.

The punctuational effect we found occurs across a range of genetic loci. If speciation is associated with small founder populations and if genetic isolation is maintained, evolutionary rates can be accelerated at potentially all loci, because the number of loci with alleles governed by drift (neutral plus nearly neutral) is increased (21). A second general mechanism for divergence is adaptive evolution as species invade new niches (22–25). Adaptive divergence is not confined to single loci. In a recent whole-genome comparison of 13,454 human genes with chimpanzee homologs, 71% diverged at coding positions, and 92% diverged at noncoding positions (26); this is in two species whose average genetic divergence among homologous genes is about 1%.

Isolating mechanisms that reduce matings between an incipient species population and the ancestral population preserve founder effects and allow adaptive divergence. There is a growing appreciation that sympatric speciation is far more common than previously believed (27) and that it arises from specific and often rapid isolating mechanisms operating in small groups. These include mutational changes to signaling molecules or behavioral pathways (28–30), pollinator switching (31), and cytoplasmic incompatibility (32). In addition to these, chromosomal rearrangements, changes of ploidy, and hybridization also produce small populations that are frequently unable to mate with the ancestral population. Comparisons of sister species that differ in ploidy suggest that evolution is often accelerated in the polyploids (33). These mechanisms could also explain the taxonomic differences we observed in the frequency of punctuational evolution, which is far more common in plants and possibly in fungi than in animals (34).

Whatever the mechanisms of the effects we have characterized, relatively rapid and punctuational bursts of evolution driven by speciation appear to make a substantial contribution to molecular divergence. By comparison, we found no molecular counterpart to the periods of stasis noted for morphological traits (1, 3, 4, 35, 36), the other half of the conventional punctuated-equilibrium description of morphological evolution. There need not be any conflict between these two observations as it is well known that molecular change can occur independently of morphology. Punctuational effects are an area of great potential for future research on

speciation combining functional-genomic, phylogenetic, physiological, behavioral, and paleontological investigations.

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Materials and Methods

Table S1

References

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ERRATUM

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Reports: “Large punctuational contribution of speciation to evolutionary divergence at the molecular level” by M. Pagel *et al.* (6 Oct. 2006, p. 119). In conducting further work to identify punctuational episodes of evolution such as reported in the paper, the authors have discovered a previously undescribed bias that affects Bayesian posterior distributions of phylogenetic trees derived from Markov chain Monte Carlo methods. The bias arises when species are closely related and thus the phylogenetic signal is difficult to detect. The bias manifests itself as a tendency in the posterior sample toward asymmetrically branching trees with short but variable branch lengths. Under these circumstances, the posterior distribution of trees can support the inference of punctuational evolution even when no such effect is present. The bias is distinct from the node-density artifact [e.g., C. Venditti, A. Meade, M. Pagel, *Syst. Biol.* **55**, 637 (2006)], and the authors will describe it in detail elsewhere. Having reanalyzed in light of this discovery the 122 phylogenetic data sets that comprise the data, the authors think that 11 may suffer from this bias, in addition to the 22 trees previously identified and removed for having node-density effects. Removing these 11 trees from the sample does not alter the conclusions. The authors find that $27 \pm 4.7\%$ of the remaining trees show the punctuational effect compared to the $35 \pm 4.8\%$ that was previously reported. They still find that the frequency of punctuational effects among plants ($43.5 \pm 10.0\%$) and fungi ($60.0 \pm 22\%$) is at least double that in animals ($18.0 \pm 4.9\%$; $\chi^2 = 7.97$, $P < 0.02$), and the asymptotic estimate of the percentage of genetic changes that can be attributed to the punctuational episodes as the tree size approaches infinity is $16 \pm 5.4\%$ as compared with $22 \pm 3.6\%$ in the original sample. The size of the punctuational effect predicts departures from a molecular clock-like mode of evolution: The correlation of $r = 0.79$ reported in Fig. 4 of the Report that measures this effect has increased to $r = 0.87$, $P < 0.0001$. The Supplementary Online Material has been modified to reflect these changes.