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Rings of single-walled carbon nanotubes

Among the most studied processes of self-organization[1,2] are the coiling and ring formation of biopolymers such as DNA and proteins. These processes are complex, involving several different types of interaction. We have found that single-walled carbon nanotubes (SWNTs), which are renowned for their extremely high flexural rigidity[3,4], can also be induced to organize themselves into rings or coils, with high yields of up to 50%. But unlike coils of biopolymers, in which hydrogen bonding and ionic interactions are usually involved, coils of nanotubes can be stabilized by van der Waals forces alone.

Scanning electron micrographs (Fig. 1a) of SWNTs with an average diameter of 1.4 nm were prepared using laser ablation[5]. The nanotubes were shortened and induced to coil by using an acid treatment with ultrasound. Transmission electron microscope (TEM) images (Fig. 1b) confirm that the rings consist of aligned ropes of SWNT. The size of the rings formed is shown in Fig. 1c.

The structure of rings (tori or coils) can be deduced from several observations. First, long SWNTs are shortened by oxidation, leaving the tube ends functional with carboxylic acid groups[6,7], arguing against the formation of a torus involving covalent bonds between carbon atoms. Images obtained by TEM and atomic force microscopy show that the rings do not have a constant thickness and height around their circumference (Fig. 1b), indicating that they may be formed by separate ropes being curled together. The rings can be taken apart and the ends of the ropes exposed (not shown), so we conclude that they are indeed produced by a coiling process.

Trace quantities (0.01 to 0.04%) of rings have previously been observed[8] and larger yields have also been claimed[9]. The rings were assumed to be perfect tori[8], stabilized by covalent bonds between carbon atoms, but our analysis suggests that they were actually coiled SWNTs.

The simplest model of the ring formation process has a SWNT coiling over itself to form a loop. Coiling involves significant strain energy because of the increased curvature, but van der Waals interactions stabilize the tubes.

The critical ring radius, R, for forming thermodynamically stable rings a few micrometres long is small, about 0.03 μm for single tubes or ropes of SWNTs 1.4 nm in diameter. According to our calculations, much lower values of R are energetically allowed than are actually observed, indicating that ring formation may be kinetically controlled. The activation energy, E_A, should be of the order of the strain energy, and $\Delta E_A \propto R^{-2}$. In our experiments, the activation energy is provided by ultrasonic irradiation. The most likely mechanism involves the hydrophobic nanotubes acting as nuclei for bubble formation and being bent mechanically at the bubble–liquid interface as a result of the bubbles collapsing during cavitation[10].

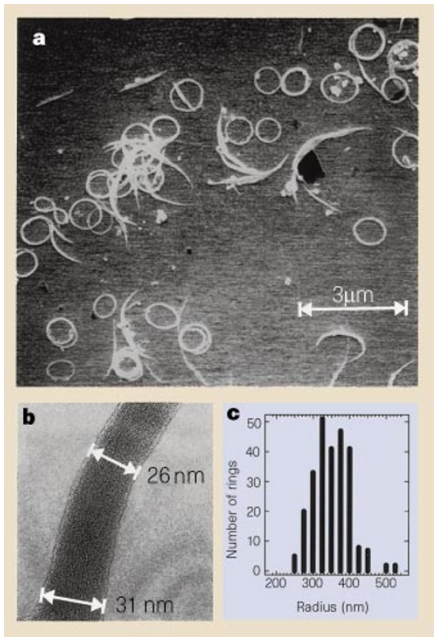

Figure 1 Ring formation in single-walled carbon nanotubes. **a,** Scanning electron micrograph of a SWNT sample dispersed on a hydrogen-passivated silicon substrate, with rings clearly visible. Rings are produced by mixing long SWNTs with a solution of concentrated sulphuric acid and hydrogen peroxide and irradiating them with ultrasound for 1–3 h (40 kHz, 190 W) at 40–50° C, which disperses them and shortens the nanotube ropes[6]. After sonication, the solution is filtered through a 0.2-μm membrane filter, and the residue dried and suspended in 1,2-dichloroethane with a brief period of sonication. A high yield requires shortening the raw nanotubes to a length of 2–4 μm. Yield varies with time of exposure to ultrasound and concentration of peroxide solution. **b,** TEM image of a section of a ring wall (courtesy of L. Gignac). **c,** Histogram showing the distribution of ring radii.

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Taxon sampling revisited

Phylogenies that include long, unbranched lineages can be difficult to reconstruct. This is because long-branch taxa (such as rapidly evolving species) may share character states by chance more often than more closely related taxa share derived character states through common ancestry[1]. Despite Kim's warning that added taxa can decrease accuracy[2], some authors have argued that the negative impact of this error, called 'long-branch attraction', is minimized when slowly evolving lineages are included to subdivide long branches[3–5]. From this they have concluded that increasing the number of species sampled per lineage results in better accuracy than increasing the number of characters per species[6]. We find, using computer simulations, that adding characters can be the more favourable strategy, even for long-branched trees, and that adding slowly evolving taxa to subdivide long branches can reduce accuracy.

An example in which adding characters is a better strategy than adding taxa is shown in Fig. 1a, which compares the performance of alternative strategies for parsimony analyses of data from a 'difficult' tree with four long external branches. This situation could occur, for example, in cases of ancient divergences — such as between fish, amphibians, lizards and mammals — where DNA sequences could be gathered

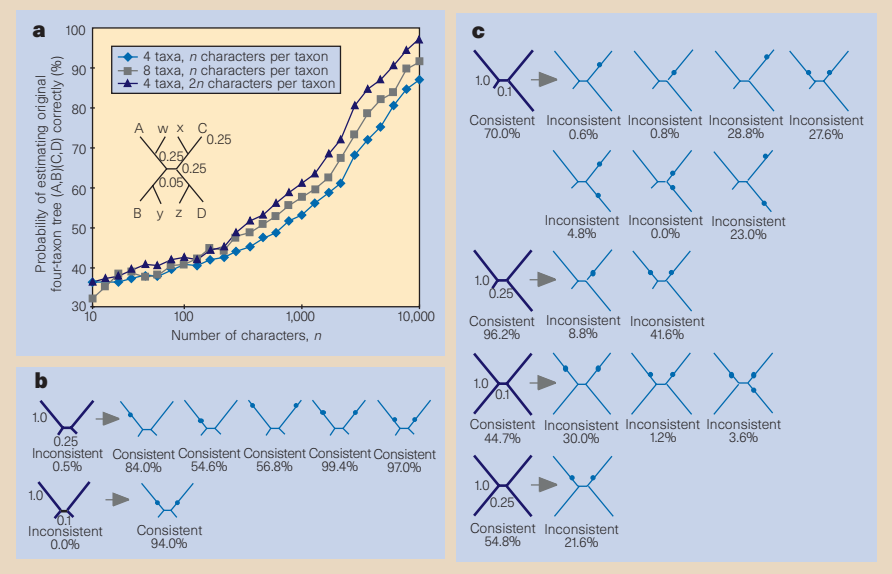

Figure 1 Modelling of phylogenetic strategies. Character data were generated on trees according to a two-state homogeneous, reversible Markov model[9]. Branch lengths are the expected number of changes per character. Trees are unrooted because determining the location of the root requires extrinsic information. PAUP version 4.0 was used for all analyses[10]. **a,** For some phylogenies that are difficult to estimate, adding characters is better for accuracy than adding taxa. Parsimony was used to estimate the relationships of A, B, C and D alone or including taxa w, x, y and z. All points are means for 1,000 simulations. **b, c,** Effect of long-branch subdivision on the accuracy of phylogeny reconstruction using parsimony, according to consistency and finite data simulation analyses. Bold indicates four-taxon trees for which adding taxa to long branches changed the estimation of relationship of those four taxa from inconsistent to consistent or vice versa. Trees on the right show the 19 (out of 288) cases where adding taxa affected the consistency of the estimation of relationships for the original four-taxon tree. The expected length of each tree is determined from the pattern frequencies predicted under the model and branch lengths. A consistent estimate is achieved if the expected length of the true tree is shorter than that of all others. Percentages refer to the number of times the correct relationships of the original four taxa were recovered in 500 replicate simulations of 5,000 characters. For trees with more than four taxa (all trees where taxa were added), characters were evolved and phylogenetic analyses were run using all taxa, after which 'added' taxa were pruned and resulting four-taxon trees were compared to the true tree. Trees shown are true (not estimated) trees. Dots show where taxa were added. **b,** Cases where long-branch subdivision caused a change from inconsistent estimation to consistency. **c,** Cases where long-branch subdivision caused a change from consistent estimation to inconsistency.

for additional species, assuming they exist, or additional DNA could be sequenced for each species. Adding either characters or taxa usually improves accuracy for original four-taxon relationships. But for the same amount of data, doubling the number of taxa with long-branch subdivision has less impact than doubling the number of characters. The best way to improve accuracy here is to increase the chance of detecting the relatively few changes occurring on the short internal branch, which is better accomplished by adding characters.

Perhaps surprisingly, adding slowly evolving taxa to subdivide long branches can sometimes actually degrade performance. To explore this possibility, we created all of the trees for four taxa composed of branches of two types: long (1.0 expected changes per character) and short (0.1 or 0.25, in separate analyses). We also created all five to eight taxon trees where a slowly evolving taxon (length 0.1) was added to subdivide one to four long branches on the original four-taxon trees. We added one to four taxa (one per branch) to bisect (0.5 from the interior node) or attach basally (0.25 from the node) or distally (0.75 from the node) on the long branch(es). We used parsimony analyses to compare whether the original four-taxon relationships were recovered more or less frequently when taxa were added. We examined consistency (whether the correct tree is obtained with increasing certainty as the amount of character data increases) and performance using a finite number of characters (5,000).

Long-branch subdivision caused changes from inconsistency to consistency (Fig. 1b) as well as from consistency to inconsistency (Fig.1c). As expected[3,6], the problems of inconsistency caused by long-branch attraction in the four-taxon case where two separated lineages are long and other branches are short (the 'Felsenstein zone') can be alleviated by long-branch subdivision. However, no other cases were found where long-branch subdivision caused a change from inconsistent estimation to consistency, and a surprisingly diverse and numerous set of conditions were found where consistently estimated four-taxon relationships were estimated inconsistently after long-branch subdivision (Fig. 1c).

The explanation for the results shown in Fig. 1c is that long-branch attraction can work for, as well as against, an investigator[7,8]. Convergent changes may cause spurious attraction between lineages, but they may also help parsimony recover relationships correctly by preventing long branches from being drawn from their proper places by other long branches. Subdividing long branches in such cases can create imbalances of homoplasy that cause the wrong long branches to attract. However, regardless of the topological result, long-branch attraction is always misleading in the sense that undetected changes will cause parsimony to misappropriate changes to internal branches. Other methods, such as maximum-likelihood and corrected-distance methods, explicitly consider undetected changes and are less likely to be affected by taxon-sampling artefacts when the assumed models are appropriate.

Our results show that the commonly recommended strategy of long-branch subdivision should not be applied uncritically. Very dense taxon sampling may yet be crucial for accuracy[4], and prudent addition of taxa is clearly beneficial in some cases[3]. However, our examples in which long-branch subdivision is harmful are just as relevant to the choice of strategy as cases in which taxa are added to alleviate long-branch attraction in the Felsenstein zone. One can imagine obtaining the top-left tree in Fig. 1c, noticing the long branches in the recovered tree, suspecting that these branches are spuriously attracting, and then adding taxa to subdivide them as a remedy. In such a case, the situation will appear to be rectified (relationships change), when in fact a problem has been created.

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