



Effect of Pruning on Dendritic Tree Topology

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(Received on 16 June 1995, Accepted in revised form on 11 July 1996)

The variability in topological shapes of observed neuronal branching patterns can accurately be described by a simple model for random sequential growth. This finding is remarkable in view of the fact that the actual neuritic growth process can vary, and includes phases of regression and removal of branches which were not considered in the model. The aim of the present study is to investigate the influence of removal of branches on the topological structure of branching patterns as well as the effect of variable growth rules.

A tree asymmetry index is used for the characterization of the topological structure of a tree. The mean value of the asymmetry index for a set of dendritic trees is sensitive to the mode of growth. The effect of removal of branches (“pruning”) on the topological structure of dendritic trees has been studied for several random pruning schemes, namely (i) removal of uniform randomly chosen subtrees, (ii) removal of uniform randomly chosen terminal segments, (iii) uniform random pruning during the growth process itself, and (iv) non-uniform random pruning schemes. It was found that the effect of pruning depends on both the mode of pruning and the mode of growth. Uniform random (terminal) pruning had no effect on the mean and standard deviation of the asymmetry index of trees grown with an order-independent mode of branching. Changes in the mean of the asymmetry index could occur either with non-uniform random pruning or when trees are grown according to an order-dependent mode of branching.

The effect of variable growth rules was studied for several specific schemes, and it could be shown that they all result in a substantial increase in the variation in the asymmetry index of the trees.

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Introduction

During development neuronal branching patterns expand in space by increasing the number and length of segments. The dynamic behavior of growth cones plays a critical role in this process. This behavior is the result of intracellular mechanisms and interactions of growth cones with their local environments via their filopodial extensions (e.g., Kater *et al.*, 1994; Letourneau *et al.*, 1994). Because of the many factors involved, growth cone propagation and branching become stochastic processes (e.g., Katz, 1985; van Veen, 1993), finally resulting in variation in the morphology of neuronal branching patterns. Despite this variability, cell classes are usually distinguishable on the basis of common morphological features that

find their origin in the specific details of the developmental process. Quantitative morphological analysis is important not only for establishing such common features but also for elucidating underlying developmental mechanisms. For this purpose mathematical modelling is very useful, and several approaches have aimed at finding minimal sets of rules for generating morphologies with specified common features. For instance, Burke *et al.* (1992) followed a *descriptive* approach in an attempt to find a parsimonious set of descriptive parameters for motoneuronal dendritic morphologies, based on the observed diameter-length relationships of segments and diameter relations at branch points. Unsuspected relationships in the original data were found, which suggested the existence of “fundamental

mechanisms" for morphological control. Van Veen & Van Pelt (1992) followed a *growth model* approach for neuronal outgrowth by modelling growth cone propagation and branching. A good correspondence was found in internal segment length relationships with those in measured morphologies of tissue cultured neurons. Woldenberg *et al.* (1993) assumed a *sequential growth process* for the addition of segments during development of rat Purkinje cell dendrites and found Fibonacci ratios between the mean lengths of topologically defined segments.

One of the main shape characteristics of branching patterns is the way in which the segments are connected to each other, called topological structure or tree type. For this characterization, metrical properties such as length and diameters of segments are not relevant. During growth, by the addition of new segments, the topological structure necessarily changes. A particular class of models for describing dendritic shape focusses only on these topological structures. Van Pelt & Verwer (1983, 1986) developed a *growth model* based on sequential branching of randomly chosen segments (QS model), as well as statistical procedures for testing observed tree types against model outcomes. Horsfield & Woldenberg (1986) and Horsfield *et al.* (1987) analysed bronchial trees, and studied the effect of sequential and of synchronous growth on the branching ratios. Kliemann (1987) followed a different (*descriptive*) approach by developing a stochastic model for the geometric characterization of dendritic structures by defining for each centrifugal order the probability of a segment to give rise to (two) daughter segments at the next order.

Using the tree asymmetry index to numerically characterize the topological structure of dendrites, Van Pelt *et al.* (1992) showed that dendritic growth according to the QS-model results in trees with a realistic topological variability (i.e., the frequency distribution of the different tree types). Excellent agreement was found with the measured topological variability of pyramidal cell basal dendrites and non-pyramidal cell dendrites in the rat visual cortex as well as for rat cerebellar Purkinje cell dendrites. Recent studies of motoneurons from several species have further established the accuracy of the QS-model in describing the topological shapes of mature motoneuronal dendrites (Dityatev *et al.*, 1995). These findings demonstrate that random sequential branching is a sufficient condition for generating the topological variability in natural neuronal branching patterns.

The QS-growth model describes only the addition of new segments during growth. The actual neuritic

growth process, however, proceeds by the dynamic behavior of growth cones, comprising not only actions as propagation and branching but also retraction and possibly the removal of complete branches. On a time-scale of minutes, periods of propagation alternate with periods of retraction (e.g., van Veen, 1993). The dynamic instability of microtubules (e.g., Horio & Hotani, 1986; Martin *et al.*, 1993), being the main cytoskeletal elements in neuritic branching structures, presumably constitutes a fundamental mechanism in this respect. Remodelling can also occur on longer time-scales, which includes the retraction of branches (e.g., Purves & Hadley, 1985; Purves *et al.*, 1986; Cline, 1991). Woldenberg *et al.* (1993) studied the number of terminal segments of rat cerebellar Purkinje cells at four different ages, and found periods of, respectively, *growth* (1–10 months), *decline* (18 months) and *regrowth* (28 months). These observations have raised serious questions about the possible effects of dendritic regression and removal of branches on the topological structure of dendritic branching patterns.

Removal of parts of dendritic branching patterns also occurs during histological practice, when neural tissue is sectioned and dendrites are analysed in single sections. Such sectioning definitely impairs the geometrical shape of the dendrites but it is not certain that it also has an effect on topology.

Using an analytical approach, van Pelt & Verwer (1984) proved that uniform random removal of subtrees, in trees grown either by the random segmental or by the random terminal growth mode, leads to tree-type probability distributions of pruned trees, which were identical to those of complete trees. Similar results were obtained by Horsfield & Woldenberg (1986) in simulation studies of the effect of random removal of terminal segments on the branching ratio of trees grown by the random segmental growth mode.

The questions addressed in the present paper are: (1) does the random removal of subtrees result in any change in topological variability?; (2) does the incorporation of pruning actions during the growth process cause topological variability to be altered?; and (3) what is the effect on the topological variance of allowing growth rules to change during dendritic tree formation? The effect of pruning has been investigated in sets of trees obtained by different growth modes, producing a full range of values for the mean topological asymmetry. Additionally, the growth process has been adapted so as to include both growth and pruning actions. This study shows that (repeated) uniform random (terminal) pruning does not increase topological variability, nor does it affect

the relationship between growth mode and expectation and variance of the tree-asymmetry index, even if such pruning actions form an integral part of the growth process itself. Only in trees produced by order-dependent branching can such pruning cause an increase in the asymmetry index. These findings demonstrate that the mean value of the asymmetry index for a set of branching patterns is a robust indicator for the topological structures of these branching patterns and, as such, for the underlying mode of growth.

In the next sections the topological aspects of branching patterns will be briefly reviewed, along with the topological growth model used. Variable growth is introduced as well as different pruning schemes and studied for their effect on topological variability.

Topological Trees

The topological structure of a three-dimensional branching pattern is defined by the number and the connectivity pattern of its segments. By ignoring all of the metrical aspects, the branching pattern reduces to a skeleton of branch points and segments in the form of a rooted tree. A distinction can be made between *root*, *branch point* and *terminal tip* and between *intermediate* and *terminal segments* [Fig. 1(a)]. The distal part of a tree, from and including a particular segment is called a *subtree*.

Different labelling schemes exist for the segments. The labelling by *degree* is based on the number of terminal segments or tips in the tree or subtree. The degree of a segment indicates the number of terminal tips in the subtree arising from that segment [Fig. 1(b)]. The labelling by *centrifugal order* is based on a centrifugal counting scheme from the root out to the tips [Fig. 1(c)]. The (centrifugal) order of a segment equals the number of branch points in the path from the root up to that segment, with root

segments having the order of zero. In a binary tree, two subtrees arise from a branch point. The degrees of both subtrees form a number pair, called *partition*, the sum of which equals the degree of the parent segment leading to the branch point. For a given number of segments, only a finite number of different topological trees (called *tree types*, see Fig. 2) exists. Multifurcations (branch points from which more than two subtrees arise) can also occur in neuronal branching patterns, but with a very low frequency (Verwer & van Pelt, 1990). Therefore, we will assume in the following that the trees are binary.

INDICES FOR THE TOPOLOGICAL STRUCTURE OF TREE TYPES

Several indices are used in the literature for describing the topological structure of trees such as the *branching ratio* (e.g., Horsfield & Woldenberg, 1986), the *mean centrifugal order* and the *tree asymmetry index* [see van Pelt *et al.*, (1989) for their respective abilities to distinguish among different tree types; also Uylings *et al.* (1989)]. In the present study we will use the tree-asymmetry index because of its favorable properties.

Partition asymmetry

This index describes the relative difference in degree r and s of the two subtrees at a branch point, defined as

$$A_p(r,s) = \frac{|r-s|}{r+s-2}.$$

Its value ranges from zero for symmetric partitions ($n/2, n/2$) to one for asymmetric partitions ($1, n-1$).

Tree-asymmetry index

This index is defined as the mean value of the partition asymmetries in a tree and ranges from zero for fully symmetric trees to a value approaching one

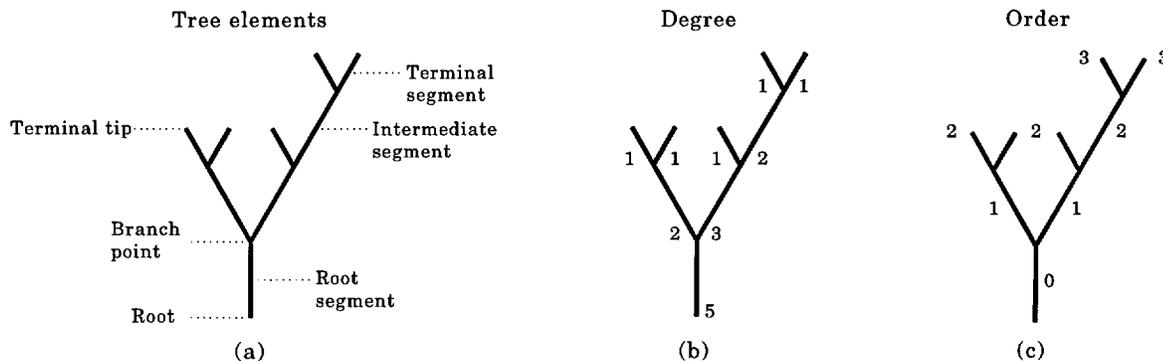


FIG. 1. (a) Elements of a topological tree. Labelling of segments according to (b) degree [i.e., the number of terminal segments in the distal part of the tree (subtree)] and (c) centrifugal order.

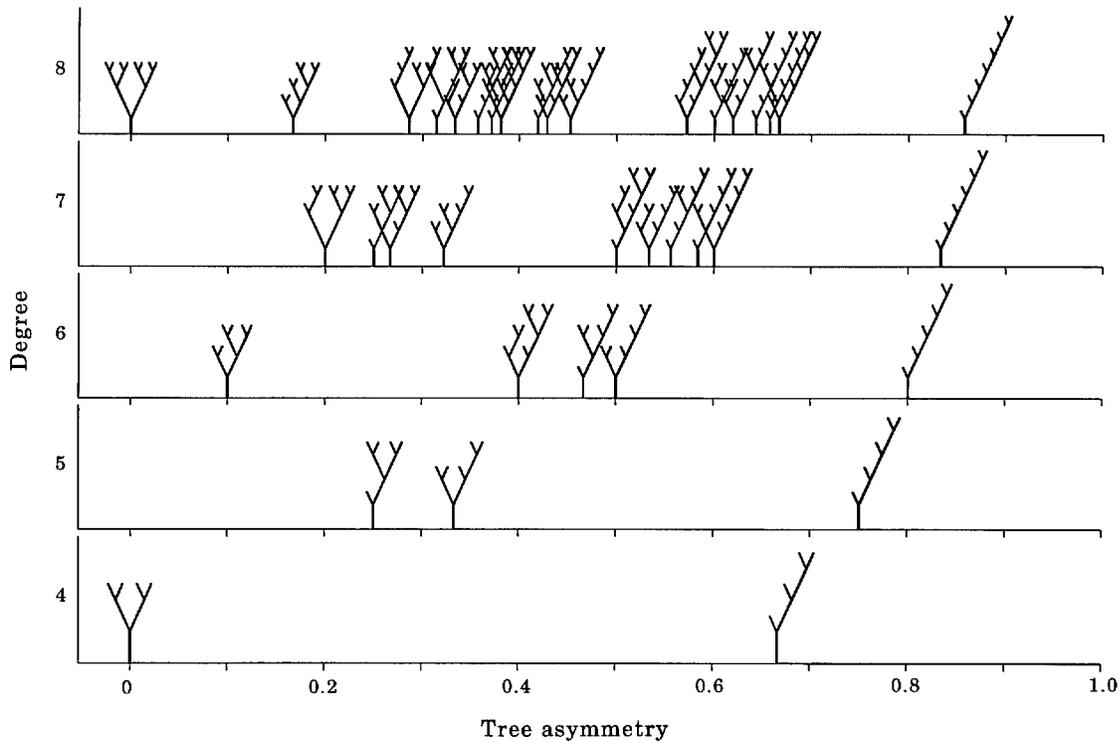


FIG. 2. Tree types with 4–8 terminal segments, plotted vs. the value for the tree-asymmetry index. The tree types are depicted such that at each branch point the largest subtree is the right one.

for asymmetric trees. The tree-asymmetry index indicates the mean relative difference in degree of subtree pairs at all the branch points in the tree (Van Pelt *et al.*, 1992).

MODELLING GROWTH OF TOPOLOGICAL TREES

A topological tree grows by branching, i.e., by the addition of new branch points and new segments (Fig. 3). Random growth indicates that the addition of branch points and segments is a stochastic process. The dimension *time* is reflected by the sequence in which the branching events occur. Sequential growth denotes a process of single branch events in succession. Synchronous growth allows more than

one branching event to occur at the same time (Horsfield *et al.*, 1987). For the present study the random sequential growth model is used.

RANDOM SEQUENTIAL GROWTH

Branching events are assumed to occur sequentially in time at randomly selected segments in the tree. During a branching event the selected segment is divided by a branch point, from which a new (terminal) segment protrudes. The random selection of segments occurs on the basis of probabilities associated with these segments. Starting with a single segment such a process of n sequential branching events eventually results in a tree with n branch points and, consequently, $n + 1$ terminal segments (assuming binary trees). Because of the randomness in the selection of branching segments, each sequence of branching events is the result of a stochastic process, as is the final formed tree. Since a finite number of different tree types exists for a given number of segments, the specific branching rules thus finally determine the probability for each tree type to occur, resulting in a tree-type probability distribution. This stochasticity is the basis for the topological variation in the trees generated by our growth model. The outcome of the branching process can also be expressed in terms of partition-probability

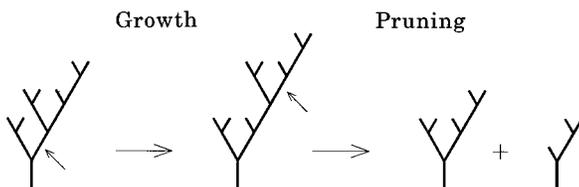


FIG. 3. Example of a growth and a pruning action of a topological tree. In a growth action, a segment (indicated by the small arrow) is replaced by two segments, separated by a branch point from which a new (terminal) segment protrudes. In a pruning action, a segment (indicated by the small arrow) and its subtree is removed from the parent tree, including the root point of the subtree.

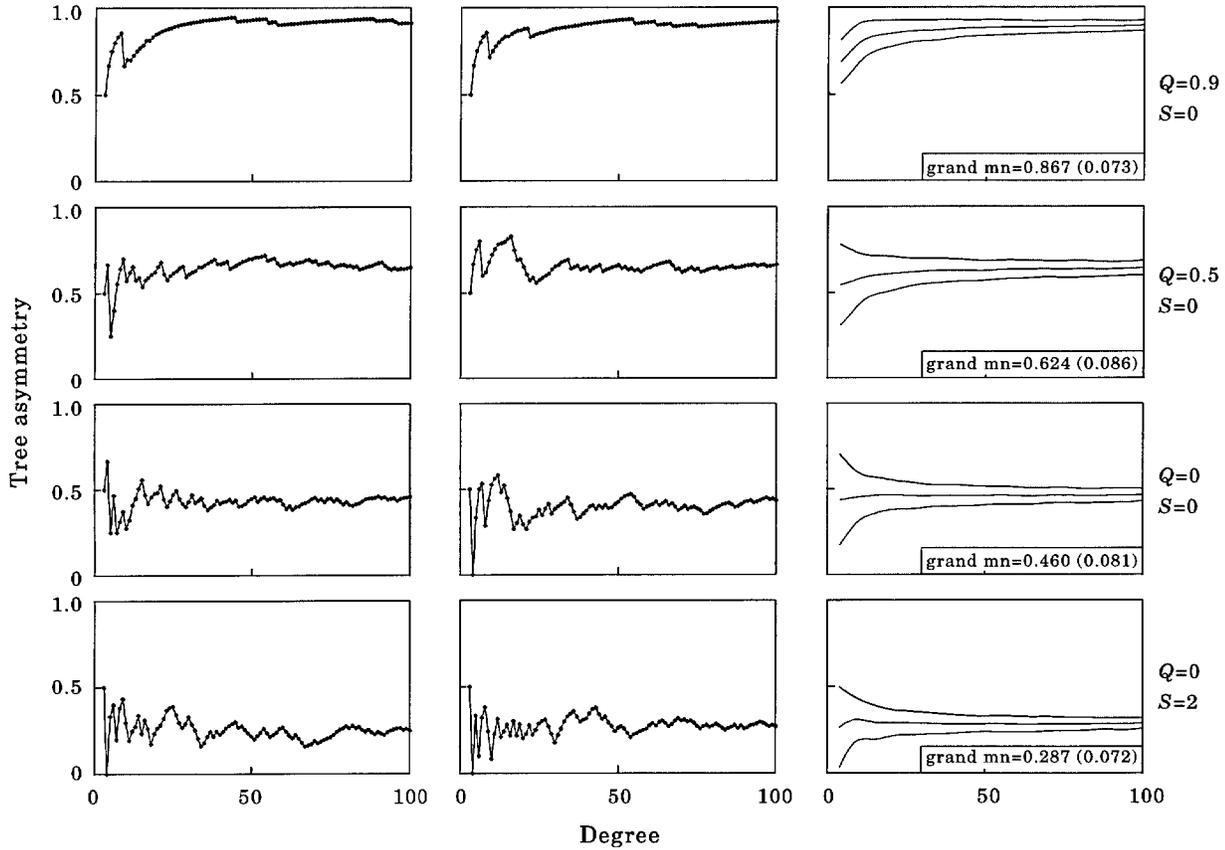


FIG. 4. Courses of the asymmetry index for growing trees, calculated for four different growth modes. The first two columns show the courses for individual growing trees. The third column shows the (smoothed) curves for the mean and standard deviation intervals for the asymmetry values, obtained by repeating the growth process 100 times, thus for populations of 100 trees per degree.

distributions, denoting how the number of terminal segments in a (sub)tree are divided over the two subtrees at its first branchpoint.

The QS model (Van Pelt & Verwer, 1986) used in this paper represents a general class of random sequential growth modes, with the branching probability of a terminal segment defined as $p_i = C2^{-S\gamma}$, and that of an intermediate segment defined as $p_i = p_i Q / (1 - Q)$. The normalization constant C ensures that the branching probabilities of all the segments in a tree add up to unity. The parameter $Q \in [0, 1)$ determines the relative contributions of intermediate and terminal segments to the branching process, while the parameter S governs the dependence on the centrifugal order γ . By varying the two parameters Q and S , a full range of probability schemes for the selection of branching segments can be defined. Two growth modes can be distinguished in particular, i.e., *random terminal growth* (*rtg*), when only terminal segments are allowed to branch with equal probability $(Q, S) = (0, 0)$, and *random segmental growth* (*rsg*), when all segments may branch with equal probability $(Q, S) = (0.5, 0)$.

VARIATION IN THE TREE ASYMMETRY INDEX

During growth a tree changes its topological structure, and thus its asymmetry index. Growth of a tree can be visualized by the course of its asymmetry index. Typical examples are given in Fig. 4 for four growth modes, with order-independent branching of both intermediate and terminal segments ($0 \leq Q < 1$, $S = 0$) or with order-dependent branching at exclusively terminal segments ($Q = 0$ [i.e., $p_i = 0$], $S > 0$). The first two columns give an impression of the variability in individual courses. The extent of the fluctuations in the asymmetry index can be calculated by repeating the growth process many times, and then calculating the mean and standard deviation of the tree-asymmetry values for each degree.

The examples in Fig. 4 show that: (1) the tree asymmetry expectation is constant during outgrowth as long as the trees are not too small; (2) this expectation is dependent on the particular mode of growth (i.e., by the values of the parameters Q and S); and (3) the standard deviation in tree asymmetry

values decreases slightly with increasing size of the trees.

A particular value for the asymmetry expectation is not uniquely associated with a certain mode of growth, but can be expected for many combinations of Q , S values. These values form contours in QS -space as is shown in Fig. 5. Roughly speaking, symmetrical trees have high probability for positive values of S and low values of Q , thus for branching of mainly proximal terminal segments. Asymmetrical trees have high probability for high Q -values and/or negative S -values, thus for branching of mainly intermediate segments and/or of distal segments. A full range of tree-asymmetry expectations can be obtained by restricting Q and S to certain areas in QS -space like the QS^+ -space ($0 \leq Q < 1$, $S = 0$ and $Q = 0$, $S \geq 0$) or the S -space ($Q = 0$, $-\infty < S < \infty$). The Q -axis will be denoted by Q -space.

Horsfield & Woldenberg (1986) used the branching ratio as an index for the topological structure, and studied the course of its value for random terminal $(Q,S) = (0,0)$ and random segmental $(Q,S) = (0.5,0)$ growth. During growth, the branching ratio followed a saw tooth like pattern (fig. 6 in their paper) illustrating how different the branching ratio behaves in comparison with the tree-asymmetry index.

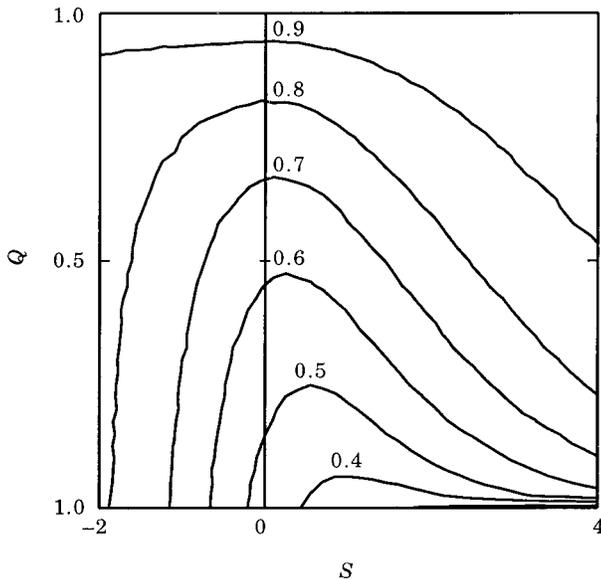


FIG. 5. Tree asymmetry, $n = 25$. Family of isoclines, connecting points in Q,S parameter space, producing equal tree-asymmetry expectations (see label at each isocline). The figure is obtained by calculating for each point of a 41×41 grid on the parameter space the mean of the asymmetry values of 2000 trees of degree 25, produced by the corresponding growth mode.

VARIABLE GROWTH MODES

Growth has been described so far as a stochastic process with probabilities determined by fixed values for the parameters Q and S . If the probability scheme for the branching segments changes during growth, it is to be expected that the growing trees will show a deviating course for the asymmetry index. Consequently, it can also be expected that the total variation in asymmetry values is larger than expected for a single (constant) growth mode.

The effect of non-stationarity in growth on tree asymmetry mean and standard deviation is illustrated for six different patterns of variable growth modes (Fig. 6). A sudden change in growth mode can result in an abrupt decrease [Fig. 6(a)] or increase [Fig. 6(b)] of the tree asymmetry expectation, or in an alternating pattern for two changes in succession [Fig. 6(c)]. The grand-mean of the asymmetry index (i.e., the mean value over the full degree range) has a value for these growth patterns which is in between the expected values for the constituting growth modes. The grand-s.d. value (i.e., the standard deviation over the full degree range) is clearly larger than the s.d. value for the constituting growth modes. Both the grand-mean value and the increase in grand-s.d. depend on the particular growth patterns, i.e., the growth modes involved and the number of growth steps for each of them.

Growth patterns in which uniform random choices for the parameters Q (within $[0,1]$) and/or S (within $[0,2]$) are made at each growth step result in a substantial increase in tree asymmetry variance, in comparison with the single growth mode expectations [Figs 6(d-f)]. For instance, in the random Q example in Fig. 6(d), with a grand-mean of 0.675, the grand-s.d. value of 0.171 is more than doubled in comparison with the s.d. value in the single growth mode (0.081) that produces the same mean asymmetry expectation [see table 3 in van Pelt *et al.* (1992)]. For the example in Fig. 6(f), the grand-s.d. value is almost 2.5 times larger than the single growth mode grand-s.d. value. These examples show that growth patterns with changing schemes for the branching probabilities of the segments result in an increased variation in the asymmetry of the tree types. For the random Q and/or S modes, the mean asymmetry index, dependent on the particular growth scheme, does not show a clear dependence on the degree, which resembles the findings for single mode curves.

Pruning of Topological Trees

“Pruning” is defined as the removal of a subtree, including its root segment, from a parent tree (Fig. 3).

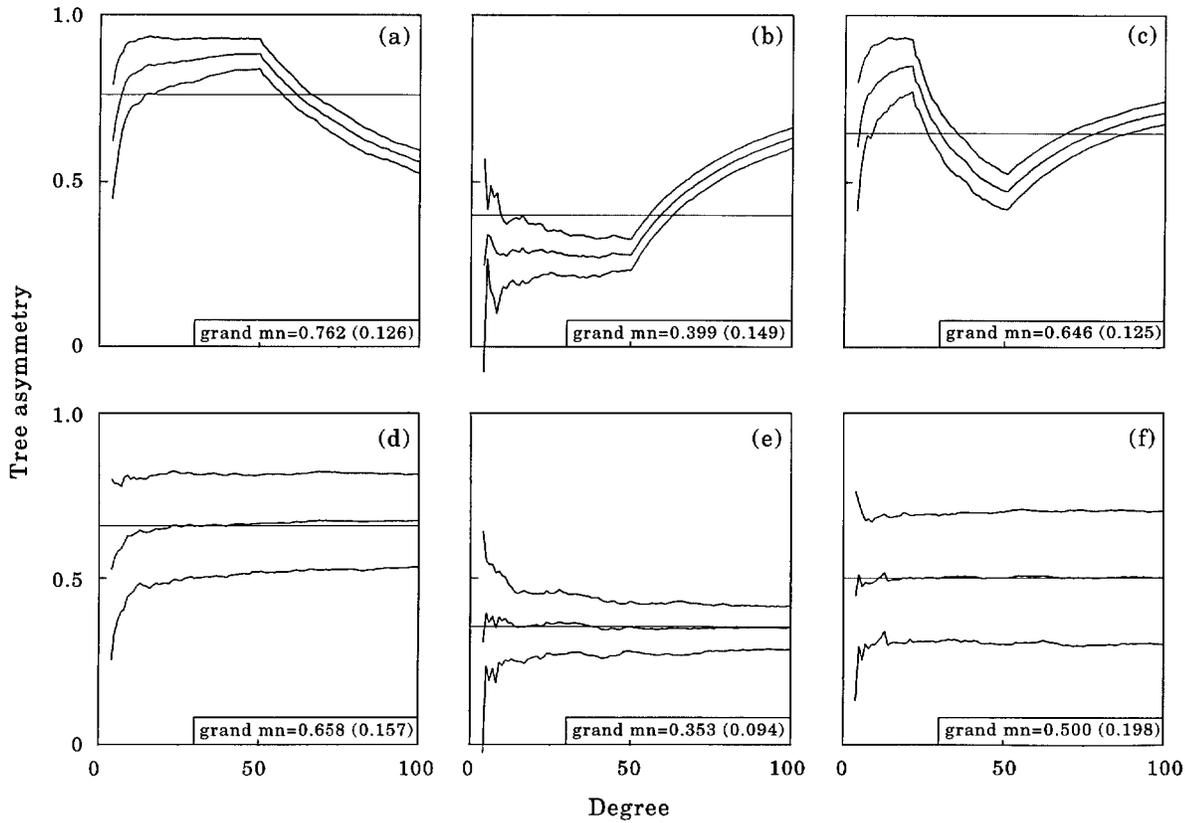


FIG. 6. Expectations of mean and standard deviation of asymmetry values of trees grown according to variable growth modes. Each figure shows the expectations obtained by repeating the growth process 100 times. The variable modes include: (a) a growth mode change from $(Q,S) = (0.9,0)$ into $(0,2)$ at degree $n = 50$; (b) a growth mode change from $(Q,S) = (0,2)$ into $(0.9,0)$ at degree $n = 50$; (c) growth modes changes from $(Q,S) = (0.9,0)$ via $(0,2)$ into $(0.9,0)$ at degrees $n = 20$ and $n = 50$, respectively; (d) uniform random choices of the parameter Q in $[0,1]$ at each growth step with $S = 0$; (e) uniform random choices of the parameter S in $[0,2]$ with $Q = 0$ at each growth step; (f) uniform random choices of both parameters Q in $[0,1]$ and S in $[0,2]$ at each growth step.

The branch point in the parent tree, having acted as the root point of the subtree is also removed. Any subtree can be removed, thus also a single terminal segment or the complete parent tree.

RANDOM PRUNING

Just as random growth was defined on the basis of the branching of segments, randomly selected according to a specified scheme of probabilities, so will random pruning be defined as the removal of subtrees, randomly selected according to a specified probability scheme.

UNIFORM RANDOM PRUNING

In uniform random pruning (*urp*), segments are selected according to a uniform probability scheme, i.e., any of the $2n - 1$ segments in a tree of degree n can be selected with equal probability of $1/(2n - 1)$. When the root segment is selected, the full tree is removed. With a probability of $n/(2n - 1)$ one of the terminal segments is selected, resulting in a pruned

tree of degree $n - 1$. When an intermediate segment of degree k is selected the pruned tree is of degree $n - k$ with $k \in [2, n]$. When a tree is repeatedly pruned it will finally disappear. This may occur already after the first (when selecting the root segment), or after several pruning actions. Two examples of *urp* sequences are given in Fig. 7. Each pruning sequence starts with a random tree of degree $n = 100$, produced by the random terminal growth mode $(Q,S) = (0,0)$. For reference, also the expected value $E(A_i)$ for the tree asymmetry and the standard deviation intervals $\sigma(A_i)$ for this mode of growth are included. The asymmetry of the original tree (with $n = 100$) of course obeys the $[E(A_i), \sigma(A_i)]$ distribution. During a pruning sequence, the degree of the tree decreases from $n = 100$ in fluctuating steps determined by the degree of the removed subtree. Termination of this process implies that the complete tree has been removed. The pruning sequences also show how the asymmetry index may change when subtrees are removed.

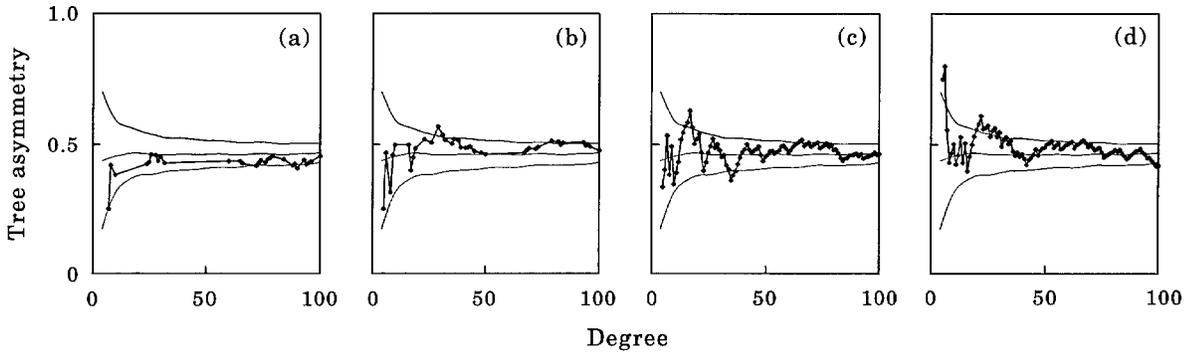


FIG. 7. Examples of uniform random pruning (*urp*) (a + b) and uniform random terminal pruning (*urtp*) (c + d) sequences. Each sequence starts with a random tree of degree 100, obtained by the random terminal mode of growth $(Q,S) = (0,0)$.

The extent to which the pruning process changes topological variability can be determined by making calculations for many pruning sequences, such that for each degree the number of pruned trees is sufficiently large to give a good estimate of the asymmetry mean and variance. The results for six different modes of growth are given in Fig. 8.

The figure illustrates a surprising correspondence in asymmetry mean and standard deviation of the pruned tree sets with the regular growth expectation.

For $(Q,S) = (0,2)$, however, the pruned trees tend to have higher asymmetry values than expected for this growth mode although the standard deviation is not increased. The effect of *urp* on topological asymmetry is most clearly demonstrated in a plot of the asymmetries of pruned trees vs. the asymmetries of regular grown trees (Fig. 9).

The data points, obtained for 13 different growth modes in QS^+ -space, are mostly positioned on the diagonal, indicating that *urp*-pruned trees of degree

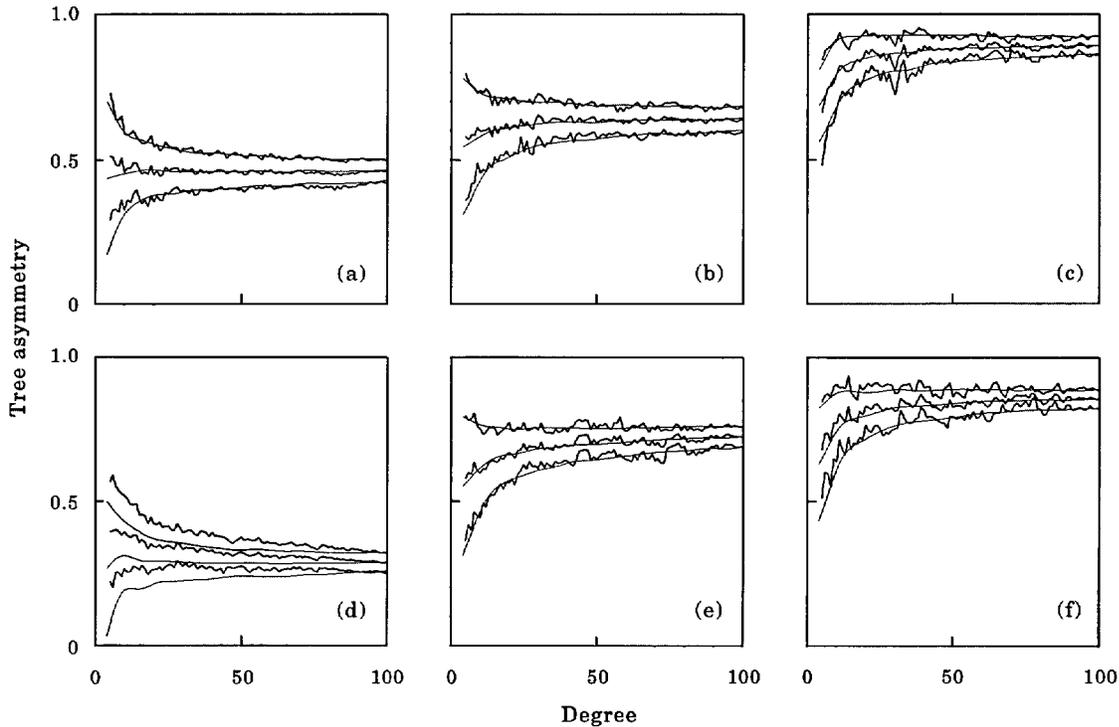


FIG. 8. Expectations of tree asymmetry mean and standard deviation of uniform random pruned (*urp*) trees. The expectations are obtained by averaging per degree the asymmetry of pruned trees from 200 different *urp* sequences, each one starting with a random tree of degree 100, produced by the indicated mode of growth. For reason of comparison, also the asymmetry expectation curves of mean and standard deviation for the regular modes of growth are displayed (thin lines). (a) $Q = 0, S = 0$; (b) $Q = 0.5, S = 0$; (c) $Q = 0.9, S = 0$; (d) $Q = 0, S = 2$; (e) $Q = 0, S = -1$; (f) $Q = 0, S = -2$.

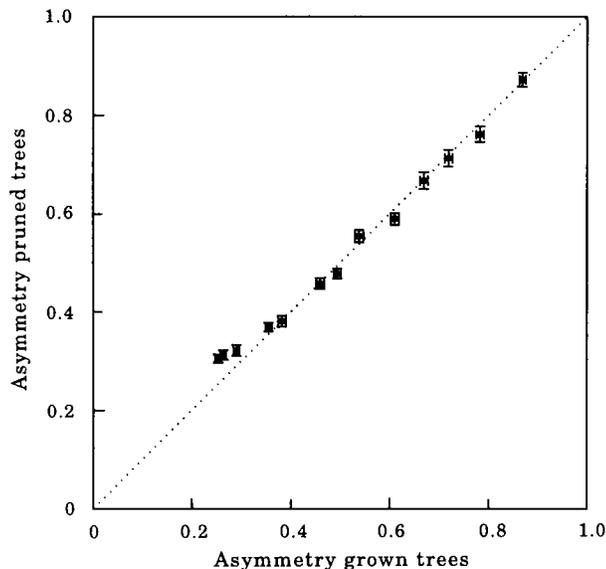


FIG. 9. Effect of uniform random pruning (urp) on the topological asymmetry. Trees are grown up to degree $n = 50$ and subsequently subjected to urp down to degree $n = 25$. The asymmetry of the pruned trees (ordinate) is plotted versus the asymmetry of regular grown trees of degree $n = 25$ (abscissa). The data points (mean and standard error) are calculated for sets of 200 trees, generated for 13 different growth modes in QS^+ -space. Most data points are positioned on the diagonal (dotted line) indicating that urp has not changed the asymmetry expectation. Some data points at small asymmetry values deviate from the diagonal, indicating that highly symmetric trees (from S^+ -space) become slightly more asymmetric during urp .

$n = 25$ (and originating from regularly grown trees of degree $n = 50$ in QS^+ -space) do not differ in asymmetry from regularly grown trees of degree $n = 25$. Some data points with small asymmetry values deviate from the diagonal, indicating that highly symmetric trees become slightly more asymmetrical during urp .

UNIFORM RANDOM TERMINAL PRUNING

Uniform random *terminal* pruning ($urtp$) proceeds by the uniform random selection and removal of a terminal segment. Removing a single terminal segment from a tree of degree n results in a tree of degree $n - 1$. Two examples of sequences of such pruning actions are given in Fig. 7. The statistical properties of the $urtp$ -pruned trees are shown in Fig. 10 for six different modes of growth.

These outcomes demonstrate that also $urtp$ does not change the mean and standard deviation of the tree-asymmetry index, but only when growth has been restricted to the Q -space. When growth has proceeded with some order dependency (i.e., with $S \neq 0$), $urtp$ appears to make the trees more asymmetrical. Thus, although trees may have similar values for their

asymmetry index, the effect of $urtp$ is differential with respect to their modes of growth, as is for instance shown in the panels of Fig. 10 for $(Q, S) = (0, -1)$ and $(Q, S) = (0.5, 0)$.

ANALYTICAL RESULTS

Analytically, it could be shown that the expectation of the asymmetry of pruned trees, obtained by urp of trees grown via random terminal growth $(Q, S) = (0, 0)$ or via random segmental growth $(Q, S) = (0.5, 0)$, is equal to the expectation for the regular growth mode (van Pelt & Verwer, 1984). This result was obtained by showing that the partition probabilities in pruned trees are equal to those in complete trees. It can be proven that the same outcome is obtained when the trees are subjected to $urtp$. The present outcomes, obtained by simulations and consistent with the analytical results, further indicate that the immunity for $urtp$ is a general property of trees grown in Q -space.

An unexpected outcome from the present simulation study is that, in addition to the mean, the standard deviation of the pruned tree asymmetries also corresponds to the expectation for the regular growth modes. This indicates that uniform random (terminal) pruning does not increase the topological variability generated by any of the regular growth modes.

NON-UNIFORM RANDOM PRUNING

In the uniform random (terminal) pruning schemes, the pruning probability of a segment does not depend on its position in the parent tree. Uniformity is lost, however, when the pruning probability becomes dependent on order or degree. To investigate qualitatively the effect of order- and degree-dependent pruning on tree asymmetry, the pruning probability of a segment (subtree) has been taken to be proportional to $2^{a \cdot \text{order}}$ or to $2^{a \cdot \text{degree}}$, respectively, with the coefficient a modulating the sign and strength of the dependency.

Order-dependent random pruning ($odrp$)

When the pruning probability increases with centrifugal order ($odrp(+)$), distal segments are the most likely ones to be pruned. This mode of pruning has a substantial effect on rtg trees, making them more symmetrical (Fig. 11). This effect disappears for increasing values of $|S|$ or Q . When the pruning probability decreases with increasing centrifugal order ($odrp(-)$), proximal segments are more likely to be pruned than distal ones. The tree will readily disappear after one or a few successive pruning actions. Any still existing pruned trees will thus have

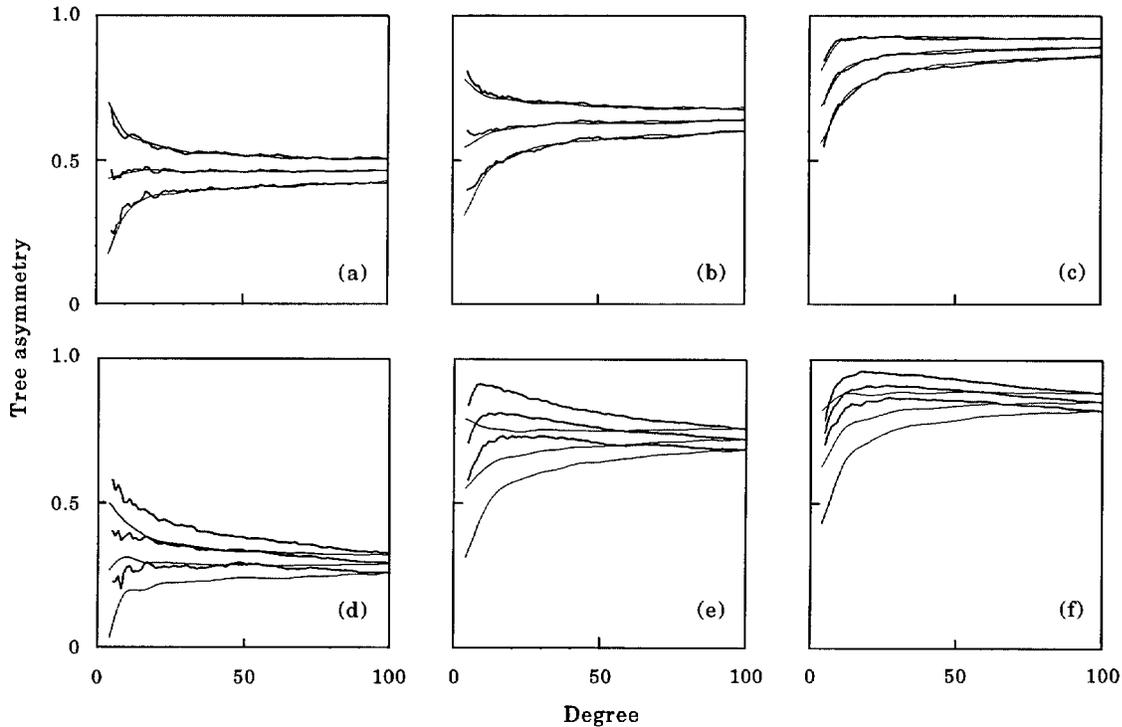


FIG. 10. Expectations of tree asymmetry mean and standard deviation of uniform random terminally pruned (*urtp*) trees. The expectations are obtained by averaging per degree the asymmetry of pruned trees from 200 different *urtp* sequences, each one starting with a random tree of degree 100, produced by the indicated mode of growth. For reason of comparison, also the asymmetry expectation curves of mean and standard deviation for the regular modes of growth are displayed (thin lines). (a) $Q = 0$, $S = 0$; (b) $Q = 0.5$, $S = 0$; (c) $Q = 0.9$, $S = 0$; (d) $Q = 0$, $S = 2$; (e) $Q = 0$, $S = -1$; (f) $Q = 0$, $S = -2$.

experienced only a few pruning actions, with little or no effect on the asymmetry.

Order-dependent random terminal pruning (*odrtp*)

When only terminal segments can be pruned, with a probability increasing with increasing order (*odrtp*(+)), the distal terminal segments are the most likely ones to be pruned. This mode of pruning has similar effect on the tree-asymmetry index as *odrtp*(+). When only terminal segments can be pruned with a probability decreasing with increasing centrifugal order (*odrtp*(-)), proximal terminal segments are most likely to be pruned. No effect was observed in S^- -space, a slight decrease in asymmetry in Q -space and an increasing asymmetry in S^+ -space.

Degree-dependent random pruning (*ddrp*)

When the pruning probability increases with increasing degree (*ddrp*(+)), large subtrees are predominantly pruned, thus causing the tree to disappear rapidly. Therefore, still existing pruned trees have experienced only few pruning actions; no effects on the asymmetry have been detected under these conditions. When the pruning probability decreases with increasing degree (*ddrp*(-)), small

subtrees and especially terminal segments are more likely to be pruned than larger subtrees. No effect was observed for *rtg* trees and only a very small effect in Q -space. However, in both S^+ and S^- space this mode of pruning makes the trees more asymmetrical. Strong degree dependency in *ddrp*(-) results in pruning of terminal segments only (degree one), making this mode similar to *urtp*.

For all these non-uniform random pruning schemes it was found that the variation in the tree-asymmetry index was not changed with respect to the regular growth mode value.

PRUNING AS A DEVELOPMENTAL RULE

Mixing growth and pruning is implemented by the following rules. The process consists of a sequence of actions each of which is randomly determined to be of a growth or pruning type according to a specified branching/pruning probability ratio. A growth action consists of the random selection of a segment according to a specified probability scheme, followed by branching of that segment. Similarly, a pruning action consists of the random selection of a segment according to a specified probability scheme with subsequent removal of its subtree. An example of

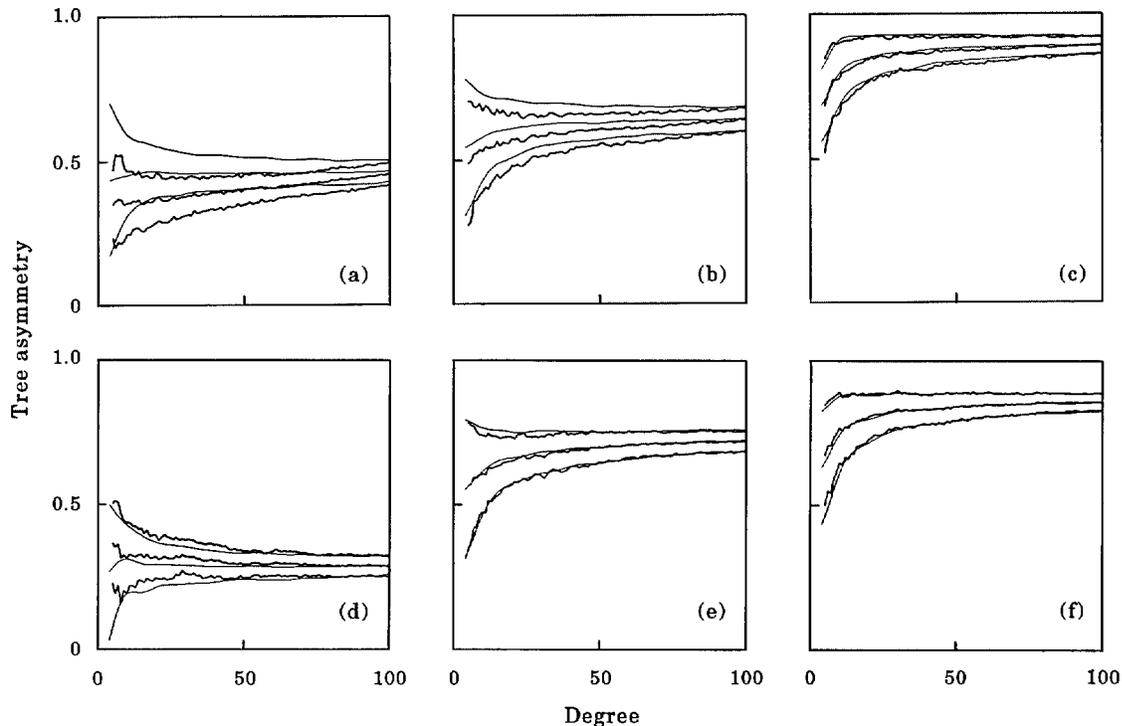


FIG. 11. Expectations of tree asymmetry mean and standard deviation of trees, subjected to order-dependent random pruning [*odrp*(1)]. The value of the exponent equals one, letting the pruning probability to increase with a factor of two for each next centrifugal order. The expectations are obtained by averaging per degree the asymmetry of pruned trees from 200 different *odrp* sequences, each one starting with a random tree of degree 100, produced by the indicated mode of growth. For reason of comparison, also the asymmetry expectation curves of mean and standard deviation for the regular modes of growth are displayed (thin lines). (a) $Q = 0, S = 0$; (b) $Q = 0.5, S = 0$; (c) $Q = 0.9, S = 0$; (d) $Q = 0, S = 2$; (e) $Q = 0, S = -1$; (f) $Q = 0, S = -2$.

such a mixed process is given in Fig. 12 in which random terminal branching is combined with uniform random terminal pruning according to a 0.6/0.4 branching/pruning probability ratio.

In this example 40% of the steps are backwards on the average, and 60% forwards in the degree axis. The

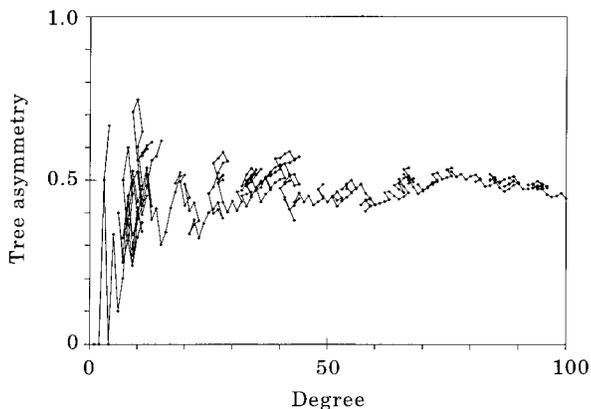


FIG. 12. Example of a mixed growth/pruning process. Each step is by chance determined to be a growth step $(Q,S) = (0,0)$ with probability of 0.6, or a *wrtpr* pruning step, with probability of 0.4.

asymmetry expectations for this process are given in Fig. 13 for six different growth modes, mixed with *wrtpr*. In Q -space similar mean (s.d.) values are produced as for the regular growth mode, as expected because *wrtpr* did not change the asymmetry expectation. In S^- -space such a mixing made the trees slightly more asymmetrical than the regular growth expectation. Surprisingly, no effect was observed in S^+ -space, although *wrtpr* alone made these trees more asymmetrical (Fig. 10).

PRUNING OF A SINGLE TREE

The asymmetry properties of pruned trees have been calculated so far by averaging over many pruning sequences, each one starting with a random tree for the chosen growth mode. However, different random pruning sequences are also obtained when they start from the same tree. Then, the variance in the asymmetry index between the sequences is initially zero, but increases during the pruning process. Two such examples are given in Fig. 14 for *wrtpr* on a random $(Q,S) = (0, -1.1)$ and a random $(Q,S) = (0.7,0)$ tree.

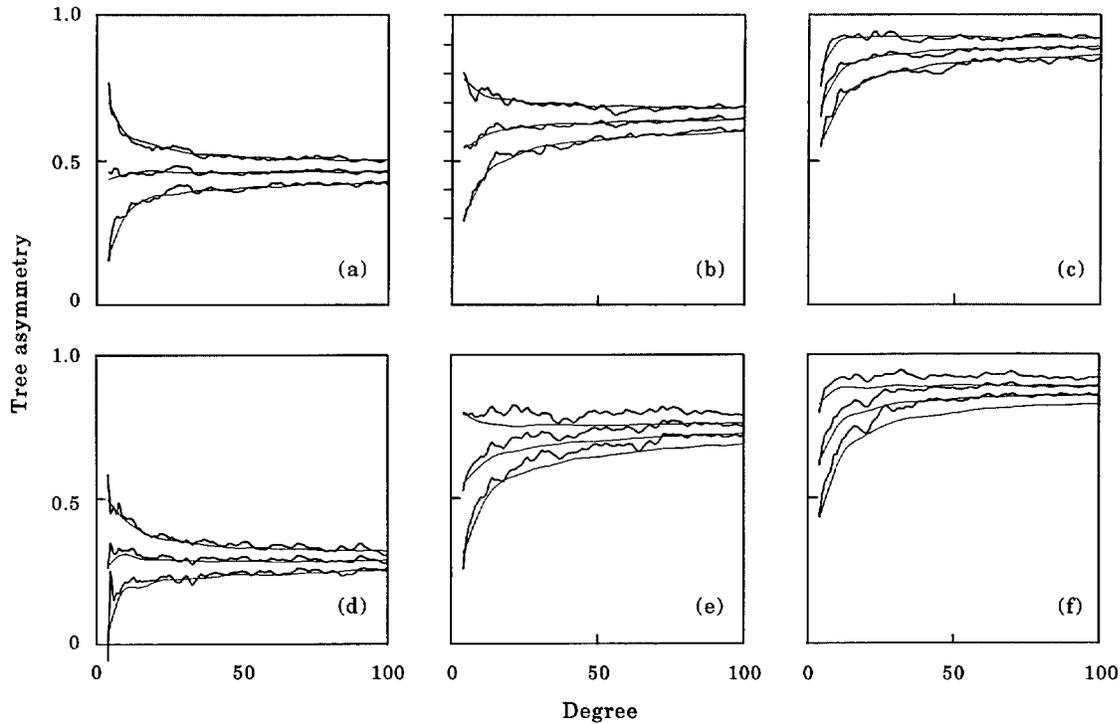


FIG. 13. Expectation of tree asymmetry mean and standard deviation of trees produced by a mixed growth/pruning process. Each sequence, starting with the tree of degree 3 [with branching code 3(1 2(1 1)), indicating iteratively the degree of a segment and, if present, the degrees of its two daughter segments (within brackets)], has a finite probability to end with complete removal of the tree. Only sequences arriving at degree 100 are used (100 in total) for the calculation of the expectations. (a) $Q = 0, S = 0$ (0.6); (b) $Q = 0.5, S = 0$ (0.6); (c) $Q = 0.9, S = 0$ (0.6); (d) $Q = 0, S = 2$ (0.6); (e) $Q = 0, S = -1$ (0.6); (f) $Q = 0, S = -2$ (0.6). All have a *urtp* pruning step with probability of 0.4.

Both trees have a similar value for the asymmetry index, but the effect of *urtp* is as differential as is found in the multiple tree calculations (Fig. 10). The implication of this finding is that a single tree may be analysed by studying the course of the asymmetry index during many *urtp* pruning sequences, thereby using *urtp* as a diagnostic tool. For instance, a tree with asymmetry index of about 0.7 may be produced

by substantial branching of either intermediate segments or distal terminal segments. When the tree is subjected to several *urtp* sequences, the course of the mean asymmetry during pruning is expected to remain constant in the first case but to increase in the second case.

Empirical Observations

TOPOLOGICAL ANALYSIS OF NEURONAL BRANCHING PATTERNS

The topological analysis of observed branching patterns proceeds by calculating the asymmetry index for each tree and obtaining the mean and s.d. for the whole population. Mean (s.d.) values were found of 0.38(0.22) and 0.43(0.26) for rat cortical pyramidal and multipolar non-pyramidal cells, respectively, and of 0.49(0.02) for rat Purkinje cells (van Pelt *et al.*, 1992). Values between 0.29(0.24) and 0.46(0.13) were found for motoneuronal dendrites from several species (Dityatev *et al.*, 1995). For these data, parameter values for Q and S were sought for which the asymmetry expectation optimally corresponded to the observed mean value. The search, restricted to

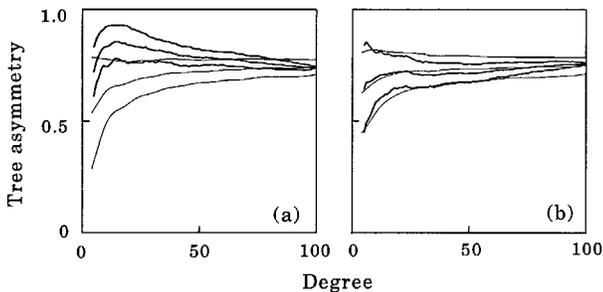


FIG. 14. Tree asymmetry mean and standard deviation, calculated from 100 *urtp* sequences, starting from (a) a single random $(Q, S) = (0, -1.2)$ tree and (b) a single random $(Q, S) = (0.7, 0)$ tree. Note the differential course of the pruned tree asymmetries for both modes of growth, for which the asymmetry expectations are included by thin lines.

TABLE 1

Mean topological asymmetry and degree of four age classes of rat cerebellar Purkinje cells

Age (month)	Number of trees	Asymmetry mean (SD)	Degree—Mean (SD) (Woldenberg <i>et al.</i> , 1993)
1	9	0.504 (0.024)	457 (19)
10	26	0.489 (0.017)	577 (66)
18	25	0.494 (0.024)	409 (90)
28	22	0.494 (0.014)	508 (55)
Total group	82	0.494 (0.020)	

Cells were obtained from R. J. Pentney. The asymmetries for the four age groups do not differ significantly. Note, that the differences in SD values result from statistical fluctuations given the number of observations per group.

QS^+ -space, resulted in (Q,S) values of (0,0.87) for the pyramidal cell group, (0,0) for the non-pyramidal cell group and (0.11,0) for the Purkinje cell group. The asymmetry expectations for these optimized growth parameters were 0.37(0.21), 0.45(0.26) and 0.49(0.02), respectively (Van Pelt *et al.*, 1992). These results showed that, for all three cell types, the model predictions of the variation in the asymmetry index agreed extremely well with the observed variability. Similar findings were obtained for motoneuron dendrites, analysed by Dityatev *et al.* (1995), who concluded that the QS model accurately predicts the observed topological variability. The search for optimal parameter values could also have been done in S -space, because both QS^+ - and S -space produce a full range of asymmetry expectations. For such a choice it is assumed that branching of intermediate segments can a priori be excluded.

The population of Purkinje cells (made available to us by R. J. Pentney), having a mean (s.d.) asymmetry of 0.494 (0.020), consisted of four age groups of 1, 10, 18 and 28 month (Woldenberg *et al.*, 1993). The mean asymmetry for each age group is given in Table I. No differences were found between the groups. During the period from 1 through 28 months, Purkinje cells experience a phase of growth from 1 to 10 months, a phase of decline at 18 months and regrowth at 28 months (Woldenberg *et al.*, 1993). During the decline phase the dendrites lost on average 336 of the total of 1153 segments (a tree of degree n has in total $2n - 1$ segments). Thus, during periods of both growth and of regression, Purkinje cell dendritic trees maintain a stable value for their asymmetry index. The s.d. values for the four age groups differ slightly from each other. Using the optimized $(Q,S) = (0.11,0)$ values for the whole population, repeated sets of model trees were generated, and the fluctuations in the obtained s.d. values determined for each age group. These s.d. fluctuations proved to correspond closely to the observed ones, indicating that they conform to the

statistics for the given number of observations per age group. These findings suggest that the growth pattern of Purkinje cells is stable because (a) the mean asymmetry index is equal for all age groups, and (b) the standard deviation for these groups is equal to the expected value for a single growth mode. The lack of change in asymmetry during the phase of regression suggests that the removal of (terminal) segments has proceeded in a uniform random way.

Discussion

The growth of branching patterns has been described by the so-called QS -model, based on the assumption of random sequential branching, with branch probabilities dependent on the *type* of the branching segment (intermediate or terminal) and on its centrifugal *order*. Each mode of growth is shown to produce trees with a particular expectation for the tree-asymmetry index. The asymmetry index A_i , of a tree is used as an indicator for the topological structure of the tree, and is defined as the mean partition asymmetry in the tree (i.e., the mean of the relative differences in the number of terminal segments between subtree pairs at all branchpoints). This expectation \bar{A}_i is equal to 0.46 for the random terminal mode of growth $(Q,S) = (0,0)$, with $\bar{A}_i < 0.46$ for S^+ -space (i.e., $Q = 0$ and $S > 0$), and with $0.46 < \bar{A}_i < 1$ for Q -space (i.e., $0 < Q < 1$ and $S = 0$) or for S^- -space (i.e., $Q = 0$ and $S < 0$). The values of \bar{A}_i are thus not uniquely associated with a particular mode of growth. According to its definition, the expectation of the tree-asymmetry index is based on the asymmetry expectations of the partitions in the trees, which in turn are determined by the partition probability distributions. Although these distributions are uniquely determined by the mode of growth, different modes may still result in similar expectations for the tree asymmetry index.

When a subtree is removed, all partitions in this subtree also disappear (as well as the partition to which the subtree belonged). Additionally, all the partitions on the path from the root to the now absent branch point will be changed. One can expect therefore, that pruning can substantially affect the asymmetry of a tree. Nevertheless, the changes appear to be small per pruning action, as is shown in Fig. 7. Large differences may occur due to repeated pruning or when the trees are small. In a statistical sense, the present study has demonstrated that uniform random (terminal) pruning does not change either the asymmetry expectations or the standard deviations of trees grown according to the Q -model (i.e., with order-independent branching probabilities). When

the trees have been grown with order-dependent branching ($S \neq 0$), however, $ur(t)p$ can change the asymmetry of trees, with urp increasing asymmetry in S^+ -space, and $urtp$ increasing asymmetry in both S^- - and S^+ -space. When the pruning process itself is non-uniform, e.g., when it depends on segment order or degree, differential effects on the asymmetry have been observed. Trees may become more symmetrical under $odr(t)p(+)$, or more asymmetrical under $odrt(-)$ in S^+ -space, or $ddrp(-)$. These findings demonstrate that the effect of non-uniform pruning on the topological asymmetry depends strongly on the mode of growth of the original trees as well as the mode of pruning. A remarkable finding is that none of the random pruning modes, studied so far, had any effect on the tree-asymmetry standard deviation.

When a pruning mode affects the asymmetry expectation for a given mode of growth, it can be expected that similar effects will occur when growth and pruning alternate randomly during the growth process. This is shown, for instance, by mixing $urtp$ with Q -space growth modes (no effect on the tree asymmetry), or with S^- -space growth modes which makes the trees more asymmetrical. Such a conclusion can not be generalized, however, because mixing $urtp$ with S^+ -space growth modes is without effect even though $urtp$ makes full-grown trees more asymmetrical.

The $urtp$ pruning scheme has a differential effect on trees grown with or without order-dependent branching, even when such modes of branching leads to similar asymmetry expectations. This differential effect occurs because of the different partition probability distributions produced by the different modes of branching, and underlying the tree-asymmetry expectation. Trees with equal mean values for their asymmetry index thus may be discriminated on the basis of their behavior under $urtp$, which then serves as a diagnostic tool for their mode of growth. Such an approach may be valuable in the analysis of the topological structure of river systems in order to discriminate between development by branching of both intermediate and terminal segments as opposed to branching of distal terminal segments only (van Pelt *et al.*, 1988).

Horsfield & Woldenberg (1986) studied the effect of pruning on the branching ratio in trees, produced by the random terminal and random segmental growth mode. The expectation of the branching ratio depends on the mode of growth but shows also a saw-tooth like pattern with increasing number of terminal segments. They found that "segmental subtraction", a pruning mode similar to $urtp$, did not change the

mean branching ratio in the case of random segmental growth, but made the trees more symmetrical in the case of random terminal growth. This last result is different from our simulated and analytical findings, and may possibly be caused by the difficulty in estimating branching ratio expectations from a saw-tooth like pattern.

Only a few empirical studies have focused on the topological implications of dendritic regression. As shown above substantial regression of rat Purkinje cell dendritic trees (Woldenberg *et al.*, 1993) did not change the mean asymmetry, suggesting a uniform random (terminal) pruning process. In an *in vitro* study of the morphological development of rat genioglossal motoneurons, Núñez-Abades *et al.* (1994) found that the first 2 weeks postnatally involved a phase of dendritic simplification with a loss of the highest-order segments and with an increase in the symmetry of the trees, consistent with our findings for $odrp(+)$. In the subsequent period new branches were formed at distal terminal segments, thereby increasing again the asymmetry of the trees. This last observation is consistent with our finding that terminal branching results in trees with higher asymmetry when distal terminal segments are more likely to branch than proximal ones. It shows that the effect of order-dependent pruning on the topological asymmetry can be compensated by subsequent order-dependent branching.

Variable growth modes

Changes in the mode of growth during outgrowth (i.e., when the probability scheme by which the segments are selected for branching is variable) result in an increase in the topological variability of the obtained trees. The extent of this increase depends on the particular growth pattern, (i.e., the growth modes involved and the number of growth steps for each of them), but may be as large as a factor 2.5 for the standard deviation. This finding is of practical use, since, when the variation in asymmetry values for an observed set of trees is larger than expected for the growth mode that produces the observed mean value, it indicates that the set of trees has probably experienced a non-stationary growth pattern consisting of different growth modes. Note, that the random pruning schemes have been shown not to increase the topological variability. The good agreement between the theoretical tree-asymmetry standard deviations and the values, found so far for the dendritic branching patterns thus suggests that they have developed according to a stationary process of branching.

Three-dimensional dendritic trees

In the present study, dendritic branching patterns are reduced to topological trees by ignoring all spatial properties. Therefore, the pruning schemes concerned only the loss of segments or subtrees, possibly dependent on topological measures like degree and order. Pruning schemes of three-dimensional dendritic structures can of course depend on spatial aspects like the length and diameters of segments or their inbedding in three-dimensional space. Regression by shortening of terminal segments and by removal of branches will evidently change these metrical properties in a manner, highly dependent on the pruning process. However, during shortening of terminal segments, topology is not changed as long as the segments do not disappear. Complete removal of terminal branches, rather than gradual shortening, is suggested to occur in Purkinje cell dendritic trees of ethanol treated rats (Pentney & Quackenbush, 1990; Pentney, 1995). Topological analysis may reveal whether such pruning occurs in an order-dependent way. A specific pruning process is the truncation of dendritic trees during histological sectioning. Then, all of the cut tips will be in the plane of sectioning and, with respect to the three-dimensional structure of the dendrite, such cutting is definitely not random. From a topological point of view, however, randomness may be preserved to a great extent because a sectioning plane does not select for a particular order or degree of segments. One might therefore argue that, when only “few” segments are cut due to sectioning, randomness ought still to be assumed. In other words, the cut trees should not be expected to deviate from the complete ones with respect to dendritic asymmetry.

I am grateful to R. J. Pentney for permission to use her data. I thank M. A. Corner for critically reading the manuscript and H. B. M. Uylings and R. W. H. Verwer for valuable suggestions.

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