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VALID PLANE TREES: COMBINATORIAL MODELS FOR RNA SECONDARY STRUCTURES WITH WATSON–CRICK BASE PAIRS*

FRANCIS BLACK[†], ELIZABETH DRELLICH[‡], AND JULIANNA TYMOCZKO[†]

Abstract. The combinatorics of RNA plays a central role in biology. Mathematical biologists have several commonly used models for RNA: words in a fixed alphabet (representing the primary sequence of nucleotides) and plane trees (representing the secondary structure, or folding of the RNA sequence). This paper considers an augmented version of the standard model of plane trees, specifically one that incorporates some observed constraints on how the folding can occur. In particular we assume the alphabet consists of complementary pairs, for instance the Watson–Crick pairs A–U and C–G of RNA. Given a word in the alphabet, a valid plane tree is a tree for which, when the word is folded around the tree, each edge matches two complementary letters. Consider the graph whose vertices are valid plane trees for a fixed word and whose edges are given by Condon, Heitsch, and Hoos’s local moves (see [C. E. Heitsch, *Combinatorics on Plane Trees, Motivated by RNA Secondary Structure Configurations*, preprint, Genome Center of Wisconsin and Department of Mathematics, University of Wisconsin–Madison]). We prove that this graph is connected. We give an explicit algorithm to construct a valid plane tree from a primary sequence, assuming that at least one valid plane tree exists. The tree produced by our algorithm has other useful characterizations, including a uniqueness condition defined by local moves. We also study enumerative properties of valid plane trees, analyzing how the number of valid plane trees depends on the choice of sequence length and alphabet size. Finally, we show that the proportion of words with at least one valid plane tree goes to zero as the word size increases. We also present some open questions.

Key words. plane trees, noncrossing matchings, RNA folding

AMS subject classifications. 05C30, 92C40

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1. Introduction. DNA has as its core a fundamental combinatorial object: words in a finite sequence, on which permutations act, with biologically significant output. Until fairly recently, RNA was considered a mute cousin of DNA, important primarily as a stepping stone. Unlike the famous double helix, RNA has a single strand. Because this strand has many nucleotides, it tends to fold onto itself like a too-long piece of tape. The base pairs created by this folding define its secondary structure. It turns out that these secondary structures (and the tertiary structures, namely the embedding of the secondary structure into 3-dimensional space) determine the functional aspects of RNA, for instance whether RNA transcribes and interprets the code in DNA, builds proteins, sends messages, or even rewrites genetic code.

In this paper we study a combinatorial model for RNA secondary structures consisting of *plane trees*, which are rooted planar trees whose half-edges are ordered counterclockwise from the root, like those shown in Figure 1. The plane tree model is well-established [4, 9, 6], and, conveniently, plane trees are one of the many sets of objects enumerated by the Catalan numbers [1, 8]. Though other sets enumerated by Catalan numbers—including noncrossing matchings and balanced parentheses—are

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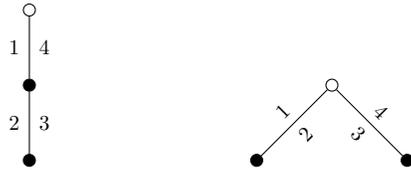


FIG. 1. The two plane trees with two edges.

also used by mathematical biologists to model RNA secondary structures, plane trees have the advantage of depicting a simplified shape of the observed RNA secondary structure.

However, the standard plane tree model does not incorporate information about base pairs. In this paper we assume an alphabet consisting of pairs of complementary letters and add the constraint that a letter can only pair with its complement. For instance, we might take the alphabet to consist of the four nucleotides $\{A, C, G, U\}$ and the complementary pairs to be the Watson–Crick complements A–U and C–G. Or, we might take the alphabet to consist of codons, each of which is a sequence of three nucleotides corresponding to an amino acid, and require that codons only bond with their perfect Watson–Crick complements.

A *primary structure* P is a word in our alphabet. Given a primary structure P , a plane tree S is called *P -valid* if folding P into the shape S leaves every letter in P matched with its complement (see Definition 2.4). Condon, Heitsch, and Hoos (see [5]) defined operations on the set of plane trees called local moves, which “unzip” two adjacent edges and “rezip” them in another arrangement (see Definition 2.2). We refine this to consider *valid* local moves, which require both initial and final plane trees to be valid plane trees in the sense depicted in Figure 2.

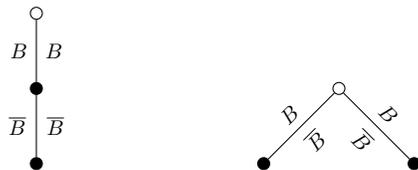


FIG. 2. Only the plane tree on the right is valid for $P = B\overline{B}B\overline{B}$.

Our basic problem has nonbiological antecedents as well. When the alphabet has exactly one complementary pair, the number of P -valid plane trees has a classical combinatorial interpretation as the number of “polite” dinner party conversations between knights and ladies of the Round Table (where “polite” encodes the notion of a noncrossing matching), as well as more serious mathematical applications to free probability theory and random matrix theory. Kemp et al. first studied this problem explicitly, giving partial results to enumerate and bound the set of P -valid plane trees for an alphabet with one complementary pair [7]. Schumacher and Yan computed elegant formulas for the number of P -valid plane trees for specific families of sequences [10].

We answer a collection of questions about primary sequences P and their P -valid plane trees. The first set assumes we are given a primary structure and asks about the possible secondary structures:

- Can we determine if a valid plane tree for P exists and, if so, find it? (Yes, by a greedy algorithm described in Definition 3.2.)

- Given a primary sequence P with at least one valid plane tree, how many orbits does it have under the action of valid local moves? (Just one, by Theorem 3.7.)
- Valid local moves of type 2 are a kind of collapsing operation on valid plane trees, defined in Definition 2.2. They allow us to create a directed graph whose vertices are the valid plane trees; for instance, there is a local move of type 2 from left to right in the plane trees in Figure 1. On which valid plane trees can a valid local move of type 2 be performed? (On all trees except the tree produced by the greedy algorithm; see Corollaries 3.6 and 3.8.)

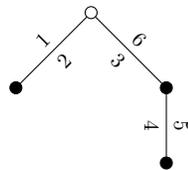
The second set of questions asks about the possible primary sequences that satisfy various constraints:

- How many sequences of length $2n$ have exactly k valid plane trees? (See results in section 4, including Remark 4.2 and Proposition 4.3, which say none if $k > C_n$ or if $C_{n-1} < k < C_n$, respectively. Also see the recent work of Wagner [11].)
- How does the collection of valid plane trees depend on the choice of sequence length $2n$ and alphabet size $2m$? (See section 4 for a preliminary study.)
- How many sequences P have a valid plane tree? (Relatively few—Theorem 5.3 shows that the ratio of sequences with a valid plane tree to all sequences approaches zero as sequence length increases.)

2. Valid plane trees and valid local moves. A plane tree is a rooted tree for which the children of each vertex are ordered. When we draw plane trees, we depict the order by arranging the children left-to-right. For example, these two trees are different plane trees:



We label the half-edges of a plane tree of size n with the numbers 1 through $2n$ starting on the left side of the leftmost edge adjacent to the root and walking around the tree counterclockwise. The edges of the tree are called $e(i, j)$, where i and j are the labels on the left and right sides of the edge, respectively. For example, this tree contains the edges $e(1, 2)$, $e(3, 6)$, and $e(4, 5)$:



In the classical plane tree model of RNA-folding, the sequence of labels on the half-edges corresponds to the primary structure. The plane tree model as presented by Condon, Heitsch, and Hoos assumed that each half-edge corresponded to a string of six G s or six C s, all of which are paired [5, section 4]. Hairpin loops, bulges, and other unmatched biological structures are omitted in the graph. (In fact, RNA secondary structure always contain unpaired nucleotides, since a hairpin loop with at least three unpaired nucleotides is needed for any pairs to bond at all.)

Plane trees are also important combinatorial objects. We note three classical properties that will be useful in our proofs.

PROPOSITION 2.1. *Properties of plane trees:*

1. (Noncrossing condition) No edges $e(i, j)$ and $e(i', j')$ have $i < i' < j < j'$.
2. For each edge $e(i, j)$ the half-edges labeled $\{i + 1, i + 2, \dots, j - 1\}$ form a subtree, as do the half-edges labeled $\{1, 2, \dots, i - 1, j + 1, j + 2, \dots, n\}$.
3. For each edge $e(i, j)$ the half-edges i and j are of opposite parity.

Proof. The noncrossing condition follows from the construction of plane trees [2, section 4.4.2]. The second property follows from the noncrossing condition, and the third property follows from the second. \square

Condon, Heitsch, and Hoos defined local moves to be the following operation on pairs of edges in a plane tree, which corresponds to an unfolding-and-refolding operation on nearby base pairs in a strand of RNA [5, Definition 8].

DEFINITION 2.2. *Let $i < j < i' < j'$, and fix a plane tree S . The two local moves are as follows:*

- *Type 1: if $e(i, j)$ and $e(i', j')$ are adjacent edges in S , then $e(i, j), e(i', j')$ are replaced by $e(i, j'), e(j, i')$.*
- *Type 2: if $e(i, j')$ and $e(j, i')$ are adjacent edges in S , then $e(i, j'), e(j, i')$ are replaced by $e(i, j), e(i', j')$.*

A local move results in a new plane tree S' .

Figure 3 shows the local moves. Note that local moves can be performed on edges without any successively labeled half-edges, namely with $i < j - 1$ and $j < i' - 1$ and $i' < j' - 1$. In other words, there can be many other edges incident to the vertices in Figure 3, including edges that come between the edges sketched in the schematic.

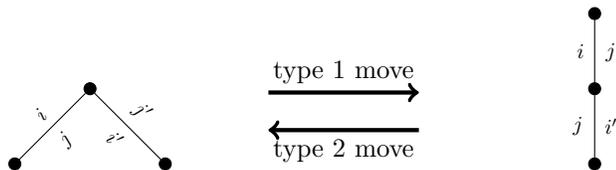


FIG. 3. Local moves.

We define plane trees for sequences other than the sequence used by Condon, Heitsch, and Hoos. We are motivated by the example of RNA, but an arbitrary alphabet consisting of complementary pairs is more mathematically natural.

DEFINITION 2.3. *A set \mathcal{A} is a complementary alphabet if for every letter $B \in \mathcal{A}$ there is a unique complement $\overline{B} \in \mathcal{A}$ that is distinct from B . If \mathcal{A} is a complementary alphabet, then taking the complement is an involution on \mathcal{A} with $\overline{\overline{B}} = B$ for each $B \in \mathcal{A}$.*

Though the collection of nucleotides $\{A, C, G, U\}$ is our foundational example, it is in fact not a complementary alphabet. The Watson–Crick pairs C-G and A-U form the strongest and second-strongest bonds, respectively. However so-called “wobble pairs” G-U also bond in nature, though more weakly than the others.

The next definition introduces one of the key objects in this paper: valid plane trees. Valid plane trees improve upon the plane tree model by considering whether,

using only perfect complements, a primary structure could actually form the secondary structure of a particular plane tree.

DEFINITION 2.4 (*P*-valid plane trees). *Let S be a plane tree with n edges, and let $P = p_1 p_2 \cdots p_{2n}$ be a word in a complementary alphabet \mathcal{A} . We say S is a P -valid plane tree if for every edge $e(i, j)$ in S the letters p_i and p_j are a complementary pair in \mathcal{A} . We may refer to S as a valid plane tree if P is clear from the context. We denote the set of all P -valid plane trees $\mathcal{V}(P)$.*

We only consider local moves that send P -valid plane trees to other P -valid plane trees. More precisely, we have the following definition.

DEFINITION 2.5. *Fix a primary sequence P , and let S be a P -valid plane tree, i.e., $S \in \mathcal{V}(P)$. A valid local move on S is a local move on S such that the resulting plane tree S' is also in $\mathcal{V}(P)$.*

The following two claims are immediate since local moves are invertible.

PROPOSITION 2.6. *A local move of type 1 is valid if and only if the inverse local move of type 2 is valid.*

PROPOSITION 2.7. *The following conditions are necessary and sufficient to guarantee the existence of a valid local move with respect to $P = p_1 p_2 \cdots p_{2n}$:*

- *There is a valid type 1 local move on adjacent edges $e(i, j)$ and $e(i', j')$ if and only if for some $B, \bar{B} \in \mathcal{A}$ the letters $p_i = p_{i'} = B$ and $p_j = p_{j'} = \bar{B}$.*
- *There is a valid type 2 move on adjacent edges $e(i, j')$ and $e(j, i')$ if and only if for some $B, \bar{B} \in \mathcal{A}$ the letters $p_i = p_{i'} = B$ and $p_j = p_{j'} = \bar{B}$.*

In fact, the property of being P -valid is inherited by subtrees in the following sense.

PROPOSITION 2.8. *Suppose that T is a P -valid tree and that T contains an edge $e(i, j)$, and denote by v the vertex of $e(i, j)$ farthest from the root.*

- *The subgraph T' induced from $e(i, j)$, together with all descendants of v , is equivalently characterized as the subgraph containing half-edges $i, i + 1, \dots, j - 1, j$.*

Define T'' to be the subgraph of T obtained by erasing T' from T . Define P' to be the subsequence of P consisting of the labels on the half-edges $i, i + 1, i + 2, \dots, j - 1, j$, and define P'' to be the subsequence of P consisting of the labels on the half-edges $1, 2, \dots, i - 1, j + 1, j + 2, \dots, n$, retaining the original indexing for notational convenience.

- *The subgraph T' is a P' -valid tree and T'' is a P'' -valid tree.*

Proof. By our convention of labeling half-edges in a plane tree, the half-edges descended from v in T are labeled $i + 1, i + 2, \dots, j - 1$. (Figure 10 shows a schematic.) The descendants of a vertex in a rooted tree form a subtree, so T' is a P' -valid tree. The graph T'' is connected by construction and is formed of the half-edges labeled $1, 2, \dots, i - 1, j + 1, j + 2, \dots, n$, so T'' is a P'' -valid tree. \square

The condition of being P -valid respects the fact that any two plane trees of size n either have some overlap or are very close to having some overlap, as the following proposition makes precise.

PROPOSITION 2.9. *Given two trees $T, T' \in \mathcal{V}(P)$, either T and T' share an edge or there exists a valid local move on one, say from T' to T'' , such that T and T'' share an edge.*

Proof. Suppose T and T' have no common edges. Suppose $e(i, i + 1)$ is a leaf of T . Since it is not a leaf in T' , the half-edges labeled i and $i + 1$ have one of the configurations in T' shown in Figures 4(a)–4(c).

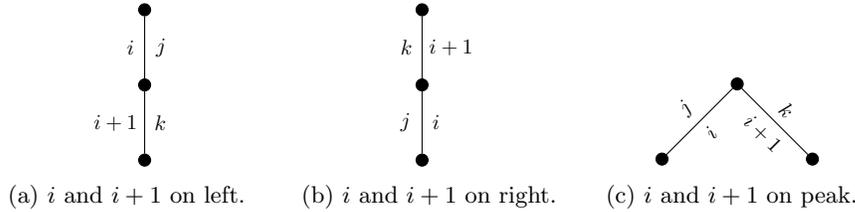


FIG. 4.

Since $e(i, i + 1)$ is an edge of a valid plane tree, the half-edges i and $i + 1$ are labeled B and \overline{B} , respectively, and so the half-edges j and k must be labeled \overline{B} and B , respectively, regardless of the configuration. Each configuration has a valid local move that results in a tree T'' with leaf $e(i, i + 1)$ as desired. \square

We can use valid plane trees and local moves to create a graph. Later sections will prove key properties of the graph defined in the next proposition. The proof follows immediately from earlier propositions.

PROPOSITION 2.10. *Fix a primary structure P . Let \mathcal{G}_P be the graph whose vertices are the elements of the set $\mathcal{V}(P)$ with an edge between two plane trees if they are connected by a valid local move. Then \mathcal{G}_P is a well-defined undirected graph. See Figure 5 for examples.*

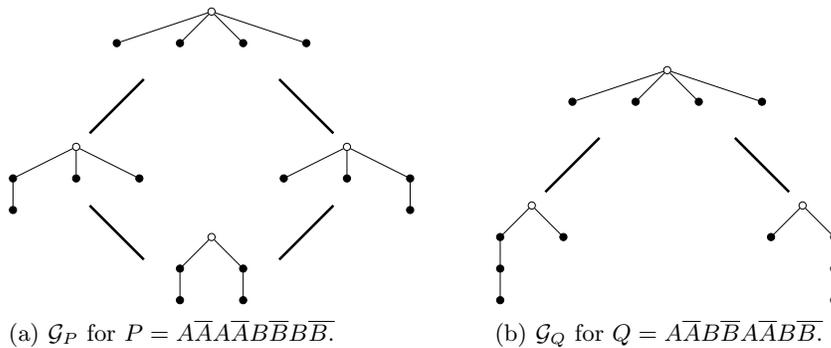


FIG. 5. Graphs of valid trees.

3. Graph of valid plane trees: Global structure. Given a primary sequence P , this section considers the overall structure of the graph \mathcal{G}_P of P -valid plane trees. We give an algorithm that produces a P -valid plane tree from P exactly when the set $\mathcal{V}(P)$ is nonempty. The algorithm is greedy in the sense that it matches letters in P opportunistically as it reads through the sequence. Theorem 3.7 shows that the graph \mathcal{G}_P is connected. Define a graph \mathcal{G}_P^+ by directing each edge in \mathcal{G}_P consistent with its type 2 move. Corollary 3.8 proves that \mathcal{G}_P^+ has a unique sink. This 2-sink is characterized in a number of different combinatorial ways in Corollary 3.11, including as the tree produced by the greedy algorithm.

Remark 3.1. We streamline proofs in this section by using the well-known bijection between plane trees with n edges and noncrossing matchings on $2n$ letters that sends each edge $e(i, j)$ in the plane tree to the ordered pair (i, j) in the matching. (The inverse of this map is obtained by writing the half-edges of a plane tree counterclockwise from the root; in each pair (i, j) the index i corresponds to a left half-edge, and the index j corresponds to a right half-edge.) Note that this map respects the poset under inclusion for plane trees and noncrossing matchings, in the sense that subtrees correspond to submatchings and vice versa. The interested reader is referred to [2, section 4.4.2].

By an extension of the classical bijection between plane trees and noncrossing matchings, every P -valid plane tree S induces a perfect matching on the letters $p_1 p_2 \cdots p_{2n} = P$ of the word P . If p_i and p_j label the two half-edges of a single edge in S , then we refer to p_i and p_j as a *pair* in the matching.

We now give the algorithm, which we describe in the language of stacks.

DEFINITION 3.2 (the greedy algorithm). *Given a primary structure $P = p_1 p_2 \cdots p_{2n}$, perform the following:*

- Push the first letter p_1 .
- For each subsequent letter p_i :
 - Peek at the stack.
 - If the letter on top of the stack is the complement of p_i , pop it.
 - If not, push p_i .

The greedy algorithm outputs a (possibly empty) stack and also a collection of ordered pairs

$$E(P) = \{(i, j) : p_i \text{ is popped at the } j\text{th step of the algorithm}\}.$$

From the biological perspective, RNA molecules do not wait patiently to be fully formed before starting to fold. As they grow longer, RNA molecules begin to refold into shapes that minimize free energy [3]. The greedy algorithm models the first folds before the effects of energy-minimization dominate.

The output of the greedy algorithm satisfies several properties that are reminiscent of Propositions 2.1 and 2.8. It creates a partial matching on each input sequence P . In fact, if the algorithm terminates with an empty stack, then it creates a perfect matching which is, moreover, a P -valid plane tree denoted $T_0(P)$. Figure 6 gives an example of this special plane tree. The following lemma is the main step in our proof that the greedy algorithm always produces a valid plane tree if any P -valid plane trees exist.

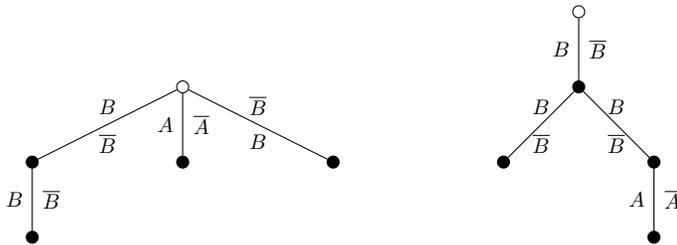


FIG. 6. Both plane trees are valid for $P = BB\overline{B}\overline{B}A\overline{A}\overline{B}\overline{B}$, but the one on the left is $T_0(P)$.

LEMMA 3.3. *Suppose that when the greedy algorithm is applied to the sequence $P = p_1 p_2 \cdots p_{2n}$ it pops p_i at the j th step. Let P' be the sequence obtained by remov-*

ing the letters $p_i p_{i+1} p_{i+2} \cdots p_{j-1} p_j$ from P and retaining (for the sake of notational convenience) the same indexing. Then implementing the greedy algorithm on P' results in the same stack as P , and moreover $E(P)$ is the disjoint union

$$E(P) = E(P') \cup M,$$

where M is a perfect matching on the set $\{i, i + 1, i + 2, \dots, j - 1, j\}$.

Proof. If p_i is popped at the j th step, then it must be at the top of the stack. Thus any of the letters $p_{i+1}, p_{i+2}, \dots, p_{j-1}$ that were pushed must also have been popped before the j th step. Since every symbol is popped at most once, none of p_1, p_2, \dots, p_{i-1} were popped in steps $i + 1, i + 2, \dots, j - 1$. This means the subset of $p_{i+1}, p_{i+2}, \dots, p_{j-1}$ that was pushed onto the stack is enumerated by the steps $\{i + 1, i + 2, \dots, j - 1\}$ at which the stack was popped or, equivalently, $E(P)$ contains a perfect matching of $\{i + 1, i + 2, \dots, j - 1\}$. It follows that the stack at the $(j + 1)$ th step on input P is the same as the stack at the $(i - 1)$ th step of input P . Since these are the same as the stack at the $(i - 1)$ th step of P' , and since P and P' share the same $2n - j$ last letters, the greedy algorithm produces the same output (pairs and stack) on P' as on P outside of $\{i, i + 1, i + 2, \dots, j - 1, j\}$. \square

Note that i labels a left half-edge in the tree $T_0(P)$ if and only if p_i was pushed onto the stack in the greedy algorithm.

In fact if a primary structure has any valid plane tree, then the greedy algorithm will terminate with an empty stack and thus produce a valid plane tree. The next theorem proves this claim using a proof by minimal counterexample.

THEOREM 3.4. *Let P be a primary structure. If $\mathcal{V}(P)$ is nonempty, then the greedy algorithm produces a valid plane tree $T_0 \in \mathcal{V}(P)$.*

Proof. If $n = 1$, then for all sequences of length $2n = 2$ that have a valid plane tree, the greedy algorithm produces the (unique) plane tree.

Consider the collection of primary sequences P such that a P -valid plane tree exists but the greedy algorithm terminates with a nonempty stack. Let $Q = q_1 q_2 \cdots q_{2k}$ be such a sequence of shortest length, i.e., Q is a sequence of length $2k$, and for all valid sequences of length 2ℓ where $\ell < k$ the greedy algorithm terminates with an empty stack.

We first claim that the greedy algorithm pops at least once. Indeed, there is at least one Q -valid plane tree which must have at least one leaf, say edge $e(i, i + 1)$. This means that $q_i = \overline{q_{i+1}}$, and so the greedy algorithm pops at the $(i + 1)$ th step, if not before.

We use this fact to produce a shorter sequence than Q for which a valid plane tree exists but the greedy algorithm terminates without an empty stack.

Suppose that the algorithm pops first at the $(j + 1)$ th step. Then it must have popped q_j off the stack. By Lemma 3.3 the greedy algorithm has the same stack when run on the sequence

$$Q' = q_1 q_2 \cdots q_{j-2} q_{j-1} q_{j+2} q_{j+3} \cdots q_{2n}$$

as when the greedy algorithm runs on Q . If $e(j, j + 1)$ is a leaf in one of the Q -valid plane trees T , then plucking $e(j, j + 1)$ off of T results in a Q' -valid plane tree. This would then contradict our hypothesis that Q was the minimal such sequence.

It remains to be shown that $e(j, j + 1)$ is indeed a leaf in one of the Q -valid plane trees. Suppose not, and consider an arbitrary $T \in \mathcal{V}(Q)$. Consider the edges containing the j and $(j + 1)$ th half-edges. They are adjacent edges that have complementary

labels since the $(j + 1)$ th step of the greedy algorithm pops q_j . By either a type 1 or a type 2 move, the tree T can be transformed into a new valid plane tree T' in $\mathcal{V}(Q)$ containing the leaf $e(j, j + 1)$ as desired. \square

We can say more about the valid plane tree that the greedy algorithm produces.

COROLLARY 3.5. *The greedy algorithm produces a valid plane tree with no valid local moves of type 2.*

Proof. Consider a valid local move of type 2 in T_0 . This local move involves two adjacent edges $e(i, j')$ and $e(j, i')$ in T_0 such that $i < j < i' < j'$. If the half-edge i has label B , then the half-edges j and j' must both be labeled by \overline{B} , while half-edge i' is labeled B . At the j th step of the greedy algorithm, p_i is on top of the stack (regardless of whether there are other edges between i and j by Proposition 2.8). So p_i must be popped, forming $e(i, j)$. By contradiction, we conclude that T_0 has no valid local moves of type 2. \square

In fact more is true: the tree T_0 produced by the greedy algorithm is the only P -valid plane tree with no valid local moves of type 2. The proof walks through the definitions, particularly using the noncrossing condition of half-edges in plane trees.

THEOREM 3.6. *Suppose T is a P -valid plane tree that is not T_0 . Then T has a valid local move of type 2.*

Proof. Consider a P -valid plane tree T that is not T_0 . They differ in some edge, so let j denote the first half-edge in which they differ; i.e., p_j labels a left half-edge in one of T or T_0 but a right half-edge in the other. We first show that p_j must label a right half-edge in T_0 . If it does not, it labels a right half-edge in T which we call $e(i', j)$. The half-edges $i' + 1, \dots, j - 1$ form a subtree of T by Proposition 2.8. This same subtree is in both T and T_0 by the hypothesis that p_j is the first label where they differ. Thus $p_{i'}$ is on top of the stack at step j in the greedy algorithm, and so $e(i', j)$ is in T_0 , contradicting our hypothesis.

Thus p_j labels a right half-edge in T_0 , say of the edge $e(i, j)$. Then

- i and j are both left half-edges in T by hypothesis;
- they have complementary labels, say B and \overline{B} , respectively, because T_0 is P -valid; and
- the half-edges labeled $i + 1, i + 2, \dots, j - 1$ form a subtree in T_0 by Proposition 2.8 and thus in T by hypothesis.

This means that T looks like the schematic of Figure 7 and thus admits a local move of type 2, as desired. \square

We now conclude that, in fact, the graph whose vertices are valid plane trees and whose edges are local moves is connected.

THEOREM 3.7. *Fix a primary structure P . The graph \mathcal{G}_P is connected.*

Proof. We induct on the length of the sequence P . The claim is trivial when P has 2 letters since in that case there is a unique valid plane tree. Suppose that for every primary structure with at most $2(n - 1)$ letters, the graph whose vertices are valid plane trees and whose edges are local moves is connected. Fix a primary structure P with n letters and consider two distinct P -valid plane trees S and T . If S and T share no common edges, then Proposition 2.9 asserts that there is a valid local move on one to a P -valid plane tree sharing an edge with the other. Without loss of generality assume that S and T both contain the edge $e(i, j)$.

Now delete the common edge $e(i, j)$. Create the subtrees S' and T' induced from

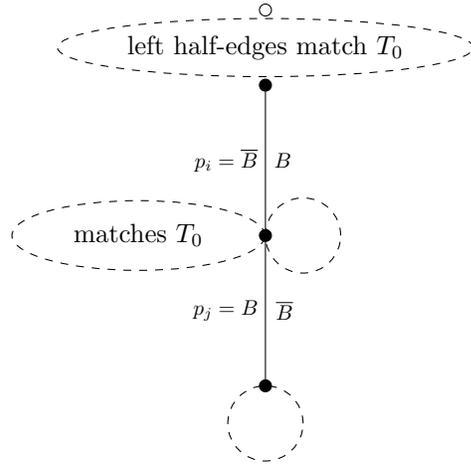


FIG. 7. If T differs from T_0 , then T has a type 2 local move.

S and T , respectively, from the edges farther from the root than $e(i, j)$ (namely, those indexed $e(i', j')$ for $i < i', j' < j$), and create subtrees S'' and T'' , respectively, from the edges closer to the root than $e(i, j)$ (namely, those indexed $e(i', j')$ for $i' < i$ and $j' > j$). By Proposition 2.8 we know that S' and T' are P' -valid plane trees for the subsequence P' of P consisting of the letters in positions i' for $i' < i$ or $i' > j$. Similarly, S'' and T'' are P'' -valid plane trees, where P'' is the subsequence of P consisting of the letters in positions i' for $i < i' < j$. By induction there are valid local moves taking S' to T' and S'' to T'' . Implement the same local moves on S to get valid local moves that take S to T . This proves the claim. \square

Furthermore, if the edges of \mathcal{G}_P are directed and correspond to type 2 local moves, then the graph has a unique sink T_0 .

COROLLARY 3.8. Fix a primary sequence P with at least one valid plane tree, and let T_0 be the valid plane tree for P produced by the greedy algorithm. Let \mathcal{G}_P^+ denote the graph whose vertices are valid plane trees for P and with a directed edge $T \rightarrow T'$ if there is a local move of type 2 from T to T' .

The valid plane tree T_0 is the unique sink in the directed graph \mathcal{G}_P^+ .

Proof. Corollary 3.5 proved that T_0 has no edges directed out, so T_0 is a sink in \mathcal{G}_P^+ . Theorem 3.6 proved that every other tree has at least one edge directed out, so there are no other sinks in the graph \mathcal{G}_P^+ . \square

Remark 3.9. The graph \mathcal{G}_P^+ may have several sources. For instance, the graph on the sequence $A\bar{A}B\bar{B}A\bar{A}B\bar{B}$ has two sources and one sink as shown in Figure 8.

COROLLARY 3.10. The graph \mathcal{G}_P^+ has no directed cycles. There is a path consisting only of type-2 valid local moves from every valid plane tree T in \mathcal{G}_P^+ to T_0 .

Proof. Consider the total distance from the root in a valid plane tree, defined as

$$d_T = \sum_{v \in T} \text{dist}(v, v_0),$$

where v_0 is the root. Figure 9 shows that d_T drops by at least one with each valid local move of type 2 (and more if the subtree labeled C in Figure 9 is nonempty).

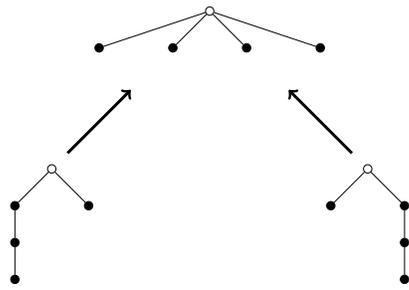


FIG. 8. \mathcal{G}_Q^+ for $Q = A\bar{A}\bar{B}\bar{B}A\bar{A}\bar{B}\bar{B}$.

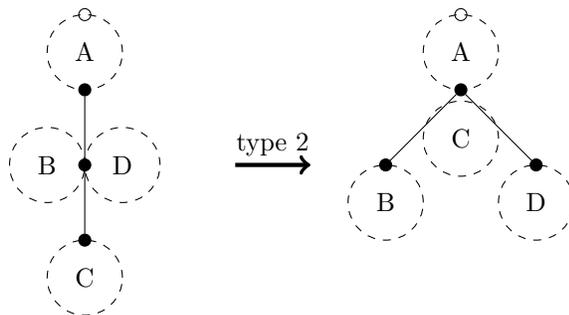


FIG. 9. A generic move of type 2.

Since the total distance d_T can only decrease, there are no directed cycles in the graph \mathcal{G}_P^+ .

Moreover Theorem 3.7 proved that the undirected graph \mathcal{G}_P is connected, and hence \mathcal{G}_P^+ has only one connected component. Consider the following process: given a tree T in the graph \mathcal{G}_P^+ , follow any edge out of T . Repeat until you reach a tree with no edges directed out within the graph \mathcal{G}_P^+ . Since \mathcal{G}_P^+ has no directed cycles and only a finite number of trees, we know this process terminates. Since T_0 is the unique tree with no edges directed out of it in \mathcal{G}_P^+ , we know the process terminates at T_0 . Thus there is a path consisting only of type 2 valid local moves from every valid plane tree T to T_0 , as desired. \square

We collect all of this information concisely as follows.

COROLLARY 3.11. *Let P be a primary sequence with at least one P -valid plane tree. The following are equivalent:*

- T_0 is the unique sink in the graph \mathcal{G}_P^+ .
- T_0 is the plane tree produced by the greedy algorithm on P .
- For each plane tree T with root v_0 define the total distance $d_T = \sum_{v \in T} \text{dist}(v, v_0)$. Among the P -valid plane trees T_0 has minimum total distance.

4. Number of valid plane trees: The size of \mathcal{G}_P . A primary sequence P has two main parameters: the length of the sequence P and the size of the alphabet from which the letters of P are drawn. The vast majority of primary sequences have no valid plane trees at all, as we discuss in the next section. This section gives results showing how the order of \mathcal{G}_P depends on n and m . It also includes open questions.

The following notation makes the discussion in this and later sections easier.

NOTATION 4.1. Given sequences of length $2n$ in complementary alphabets with $2m$ letters:

- $\mathcal{P}(n, m)$ is the set of valid sequences of length $2n$ in a complementary alphabet with $2m$ letters, namely, sequences P with at least one P -valid plane tree.
- $N(n, m, k)$ is the set of valid sequences $P \in \mathcal{P}(n, m)$ such that $|\mathcal{V}(P)| = k$.
- $R(n, m)$ is the set of $k \in \mathbb{Z}$ such that $N(n, m, k)$ is nonempty.

4.1. **Describing $N(n, m, k)$.** We can bound the k for which $N(n, m, k)$ is nonzero and can describe $N(n, m, k)$ in the boundary cases. We have the following initial observations, which are generalizations of observations made by Kemp et al. [7, Proposition 1.7].

Remark 4.2. A sequence obtains the maximum number of valid plane trees in the following circumstances:

- A primary sequence P of length $2n$ has at most C_n valid plane trees, where C_n is the n th Catalan number.
- A primary sequence P has exactly C_n valid plane trees if and only if P is of the form $B\bar{B}B\bar{B}\dots B\bar{B}$.
- Using an alphabet of size $2m$, there are $2m$ such sequences, namely, $|N(n, m, C_n)| = 2m$.

If a sequence in $\mathcal{P}(n, m)$ has more than C_{n-1} valid plane trees, then all C_n possible plane trees must be valid. In other words, $R(n, m)$ has a large gap between its largest element C_n and its second largest element C_{n-1} . Our argument uses the recurrence relation for Catalan numbers on a sequence that is not $(B\bar{B})^n$.

PROPOSITION 4.3. Fix $k > 0$. If a sequence P of length $2n$ has k valid plane trees and $k \neq C_n$, then k is at most C_{n-1} .

Proof. Let $P \in \mathcal{P}(n, m)$ have exactly k valid plane trees with $k < C_n$. For each p_i in P , the half-edge i can only be paired with j if $p_j = \bar{p}_i$ and j is of the opposite parity to i . Suppose P contains the letter B in exactly $\ell_B > 0$ odd-indexed places. Since there is at least one P -valid plane tree, there must be ℓ_B even-indexed places containing the letter \bar{B} . In any valid tree $T \in \mathcal{V}(P)$ these ℓ_B edges can, if adjacent, interact via local moves and thus form at most C_{ℓ_B} valid subforests. This is true for any letter in \mathcal{A} , so the set $\mathcal{V}(P)$ has at most $k = \prod_{B \in \mathcal{A}} C_{\ell_B}$ valid trees.

Let B_1 denote the letter p_1 . The product $C_a \cdot C_b$ is less than or equal to C_{a+b} for any $a, b \geq 0$ by the recurrence relation defining Catalan numbers. Moreover, each $\ell_B \leq n - 1$ since $P \neq (B\bar{B})^n$ by hypothesis. This means

$$k \leq \prod_{B \in \mathcal{A}} C_{\ell_B} \leq C_{\ell_{B_1}} \cdot C_{n-\ell_{B_1}}.$$

The product of Catalan numbers $C_i \cdot C_{n-i}$ is maximized when $i = 1$ or $i = n - 1$. Thus

$$C_{\ell_{B_1}} \cdot C_{n-\ell_{B_1}} \leq C_1 \cdot C_{n-1} = C_{n-1}. \quad \square$$

We can also characterize the sequences P that achieve the bound of Proposition 4.3.

COROLLARY 4.4. If a sequence $P = p_1 p_2 \dots p_{2n}$ has exactly C_{n-1} valid plane trees, then

- all but one of the odd-indexed p_i are the same letter;
- all but one of the even-indexed p_i are the same letter; and

- if for i and j of opposite parity p_i and p_j differ from the other odd- and even-indexed letters, respectively, then either
 - one of i, j is 1 and the other is $2n$, or
 - $|i - j| = 1$.

Proof. By the previous proof, if more than two letters in \mathcal{A} appear as p_i for i odd, then $|\mathcal{V}(P)|$ will be strictly less than C_{n-1} . If exactly two letters appear in odd-indexed positions, say A and B (possibly complements), then there are at most $C_{\ell_A} \cdot C_{\ell_B}$ P -valid plane trees. By hypothesis there are C_{n-1} total P -valid trees, so without loss of generality $\ell_A = n - 1$ and $\ell_B = 1$. Thus all but one odd-indexed p_i are A and, because P is valid, all but one even-indexed p_i are \bar{A} .

Let p_i and p_j be the odd-indexed letter that differs from A and the even-indexed letter that differs from \bar{A} , respectively. We assume that $i < j$ but not that i is the odd index. Then any P -valid plane tree contains the edge $e(i, j)$ according to the schematic in Figure 10.

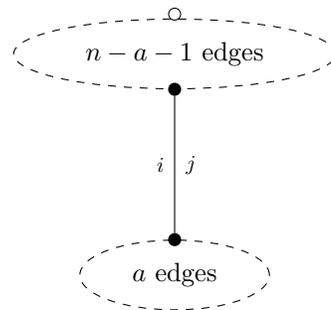


FIG. 10. Edge $e(i, j)$ partitions a valid plane tree into valid subtrees.

There are $C_a \cdot C_{n-a-1} = C_{n-1}$ possible P -valid plane trees, so either $a = 0$ and $e(i, j)$ is a leaf, or $a = n - 1$ and $e(i, j)$ is the unique edge incident to the root, as claimed. \square

The next corollary follows immediately by counting possible locations and letters for A and B .

COROLLARY 4.5. *There are $2m(2m - 1)(2n)$ sequences $P \in \mathcal{P}(n, m)$ with exactly C_{n-1} valid plane trees, i.e.,*

$$|N(n, m, C_{n-1})| = 4m(2m - 1)n.$$

We can characterize the set $N(n, 1, 1)$ using a similar argument.

PROPOSITION 4.6. *The set $N(n, 1, 1)$ has size $2n$.*

Proof. We show that, unless the primary sequence P on the alphabet $\{B, \bar{B}\}$ has a very special form, we can find a P -valid plane tree with a valid local move. This would imply that $\mathcal{V}(P)$ has more than one element and thus that $P \notin N(n, 1, 1)$.

Suppose v is a vertex in a P -valid plane tree on the alphabet $\{B, \bar{B}\}$ that is incident to three edges. Then two of the left-edges incident to v have the same label by the pigeonhole principle. Thus there is a valid local move at the vertex v . It follows that if P has only one P -valid plane tree, then that tree is a path. The root may be anywhere on that path. To prevent local moves, all edges to the left of the root must have the same letter on their left half-edges, while edges to the right must have that

label's complement on their left half-edges. There are two choices for the side that has B and n choices for the location of the root. Thus there are $2n$ primary sequences P with $|\mathcal{V}(P)| = 1$. \square

Without loss of generality, all such sequences P must have the form $P = B^k(\overline{B})^n B^{n-k}$. Heitsch and Poznanović [4] used similar arguments to prove that if T is a plane tree in which the maximum degree of a vertex is d , then there exists a sequence $P \in N(n, m, 1)$ with T as its unique valid tree if and only if $m \geq \lceil \frac{d}{2} \rceil$. These arguments lead us in two directions.

Question 4.7. Consider $N(n, m, 1)$ for $m > 1$. The previous argument can be modified to show that any plane tree with a vertex of degree at least $2m + 1$ cannot represent a primary sequence for $P \in N(n, m, 1)$.

- Can we characterize the primary sequences $P \in N(n, m, 1)$?
- Given a plane tree T can we characterize the sequences $P \in N(n, m, 1)$ such that $\mathcal{V}(P) = \{T\}$?

A related question, about the total number of possible secondary structures, can be addressed using Motzkin numbers, as described in [4]. However, that enumeration does not count the number of sequences that have only one secondary structure.

4.2. Comparing $R(n, m)$ for different n and m . Proposition 4.3 can be interpreted as showing that $|R(n, m)| \leq C_{n-1} + 1$. We further analyze the sets $R(n, m)$ and exploit prime factorization to construct sets $R(n, m)$ that contain desired elements.

PROPOSITION 4.8. *Let $k \in \mathbb{Z}_{>0}$ factor into a product of primes $k = k_1^{\alpha_1} \cdots k_\ell^{\alpha_\ell}$ such that for each i , there exists a pair (n_i, m_i) with $k_i \in R(n_i, m_i)$. Then*

$$k \in R\left(\sum_{i=1}^{\ell} \alpha_i n_i, \sum_{i=1}^{\ell} \alpha_i m_i\right).$$

Proof. Fix a complementary alphabet with $2 \sum_{i=1}^{\ell} \alpha_i m_i$ letters. For each i with $1 \leq i \leq \ell$ let P_i be a primary sequence in $N(n_i, m_i, k_i)$. Such a P_i exists since $k_i \in R(n_i, m_i)$. For each i make α_i copies of P_i and denote them $P_{i,1}, P_{i,2}, \dots, P_{i,\alpha_i}$. Now change the complementary letters as needed so that no two primary sequences $P_{i,j}, P_{i',j'}$ share a letter unless $i = i'$ and $j = j'$.

Consider the sequence $P = P_{1,1}P_{1,2}P_{1,3} \cdots P_{\ell,\alpha_\ell}$ obtained by concatenating these sequences. No half-edge from $P_{i,j}$ can match a half-edge from $P_{i',j'}$ unless $i = i'$ and $j = j'$ since the alphabets used for the subsequences $P_{i,j}$ and $P_{i',j'}$ are distinct. Thus every P -valid plane tree consists of $P_{i,j}$ -valid plane subtrees joined at a common root, and the only P -valid local moves are $P_{i,j}$ -valid local moves on the subtrees. Since there are k_i of the $P_{i,j}$ -valid plane trees, and since the $P_{i,j}$ -valid local moves are independent, there are $k = k_1^{\alpha_1} \cdots k_\ell^{\alpha_\ell}$ trees in $\mathcal{V}(P)$, as desired. \square

Wagner proved a conjecture of an earlier version of this paper that for all $k \in \mathbb{Z}_{>0}$ there exists n, m such that $k \in R(n, m)$. In fact, he showed the stronger result that every integer k appears in $R(n, 1)$. He also proved that $R(n, m)$ is not generally the same set as $R(n, 1)$, which disproved another conjecture of ours [11].

5. How many sequences are valid? This last section enumerates the total number of primary sequences that have *any* valid plane trees. These sequences are extremely unusual, in the sense that the ratio of sequences that are valid in a fixed alphabet approaches zero as sequence length increases.

Our main tool comes from Corollary 3.11: every primary sequence P corresponds to at most one labeled tree with no valid local moves of type 2, namely, the tree T_0 produced by the greedy algorithm. Thus we may count the number of valid primary sequences by counting the number of labeled plane trees with no type 2 local moves.

LEMMA 5.1. *Every plane tree T is the output $T_0(P)$ of the greedy algorithm for some valid sequence P .*

Proof. Given a plane tree T , label the edges incident to the root with any complementary pair from the alphabet. Each edge not incident to the root has a parent edge. Starting from the edges adjacent to the root, label each edge so that it has no local move with its parent edge. Then the labeled tree T has no local moves of type 2 and thus is the output $T_0(P)$ of the greedy algorithm for the sequence P obtained by reading the labels on the half-edges. \square

Note that a labeled tree generated by this procedure does not produce a tree with *no* local moves but rather only with none of type 2. For example, if a plane tree has a vertex of degree $2m + 1$ or more, there is always a local move; the process in the previous lemma simply ensures that it is a local move of type 1.

We can enumerate valid sequences by counting labeled trees with no local moves of type 2.

THEOREM 5.2. *The number of valid primary sequences is*

$$(1) \quad |\mathcal{P}(n, m)| = \sum_{\substack{\lambda=(\lambda_1, \dots, \lambda_k) \\ \text{a composition of } n}} (2m)^k \cdot (2m - 1)^{n-k} \cdot \prod_{i=1}^k C_{\lambda_i-1},$$

where C_{λ_i-1} is the $(\lambda_i - 1)$ th Catalan number.

Proof. By Lemma 5.1 we can count the number of valid sequences by counting the number of plane trees with no local moves of type 2. We partition the set of plane trees based on the number of edges incident to the root and the size of the subtrees coming off of those edges.

Suppose there are k edges incident to the root. The k subtrees descending from those k edges divide the n edges of the tree into k parts, say with the i th part of size λ_i including the edge incident to the root. This gives a composition $\lambda = (\lambda_1, \dots, \lambda_k)$ of n . Moreover the i th subtree can be any of C_{λ_i-1} possible plane trees.

Label the edges in order of their distance to the root. Each of the k edges incident to the root has no parent edge and thus has $2m$ possible labels. Every other edge in the tree has a parent, so there are $2m - 1$ ways to label it without creating a local move of type 2. Thus there are $2m \cdot (2m - 1)^{\lambda_i-1} \cdot C_{\lambda_i-1}$ possibilities for labeling the i th subtree.

Taking the product over all k such subtrees, there are $(2m)^k \cdot (2m - 1)^{n-k} \cdot \prod_{i=1}^k C_{\lambda_i-1}$ labeled plane trees with no valid local moves of type 2 corresponding to the composition λ . Summing over all compositions λ of n gives the desired result. \square

The other main result of this section is that the ratio of valid primary sequences to all primary sequences approaches zero as n increases.

THEOREM 5.3. *Fix m , and let $S(n, m)$ denote all possible words of length $2n$ over a complementary alphabet of size $2m$. Then*

$$\lim_{n \rightarrow \infty} \frac{|\mathcal{P}(n, m)|}{|S(n, m)|} = 0.$$

Proof. When $m = 1$, the number of valid plane sequences $|\mathcal{P}(n, 1)|$ is at most $\binom{2n}{n}$, since each valid plane sequence must have the same number of letters B as \overline{B} . (In fact, $|\mathcal{P}(n, 1)| = \binom{2n}{n}$.) There are a total of 2^{2n} words in $\{B, \overline{B}\}$ of length $2n$. By Stirling's approximation for $n!$ we know that

$$\lim_{n \rightarrow \infty} \frac{\binom{2n}{n}}{2^{2n}} = \lim_{n \rightarrow \infty} \frac{4^n}{\sqrt{\pi n} 2^{2n}} = \lim_{n \rightarrow \infty} \frac{1}{\sqrt{\pi n}} = 0.$$

When $m > 1$, we obtain an upper bound on $|\mathcal{P}(n, m)|$ by overcounting the set of valid plane trees T_0 that can be produced by the greedy algorithm. The greedy algorithm produces different outputs on different valid sequences because there is only one way to read the sequence off of the labeled plane tree. We use the total number of valid plane trees as our upper bound; this is a strict overcount because some valid plane trees have a valid local move of type 2, unlike output of the greedy algorithm. There are $(2m)^n C_n$ valid plane trees, since there are C_n plane trees with n edges, $2m$ ways to pick the letter labeling each left half-edge, and 1 way to pick the complementary letters on the right half-edges. There are a total of $(2m)^{2n}$ words in the alphabet of length $2n$. Thus

$$\lim_{n \rightarrow \infty} \frac{|\mathcal{P}(n, m)|}{|S(n, m)|} < \lim_{n \rightarrow \infty} \frac{(2m)^n C_n}{(2m)^{2n}} = \lim_{n \rightarrow \infty} \frac{1}{n+1} \frac{\binom{2n}{n}}{(2m)^n} \leq \lim_{n \rightarrow \infty} \frac{1}{n+1} \frac{\binom{2n}{n}}{2^{2n}}$$

using the definition of Catalan numbers and the fact that $m \geq 2$. The limit of the ratio $\frac{\binom{2n}{n}}{2^{2n}}$ was just computed to be zero, so as n grows we obtain

$$\lim_{n \rightarrow \infty} \frac{|\mathcal{P}(n, m)|}{|S(n, m)|} < \lim_{n \rightarrow \infty} \frac{1}{n+1} \frac{\binom{2n}{n}}{2^{2n}} < \lim_{n \rightarrow \infty} \frac{\binom{2n}{n}}{2^{2n}} = 0$$

which proves the claim. \square

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