Spiral phyllotaxis of needle fascicles on branches and scales on cones in *Pinus contorta* var. *latifolia*: Are they influenced by wood-grain spiral?

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Abstract: Neither the relationship between the chirality of spiral phyllotaxis and spiral wood grain nor the cause or ontogeny of such a relationship has been examined previously. To this end, chirality of the spiral in phyllotaxis of needle fascicles and cone scales were contrasted with wood-grain spiral in seedlings, young, and mature trees of Rocky Mountain lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) in central British Columbia. To assess chirality of phyllotaxis, the relationship between numbers of contact parastichies and chirality of phyllotaxis in scales on cones and needle fascicles on branchlets was determined. Three or 8 clockwise acropetal contact parastichies were indicative of a clockwise generative spiral, while totals of 2, 5, or 13 clockwise acropetal parastichies were indicative of a counter-clockwise generative spiral. Lodgepole pine trees were nearly always chimeric, i.e., having clockwise and counter-clockwise phyllotaxis on the same individual, but there was a high overall correspondence between the chirality of phyllotaxis in cone scales and subtending needle fascicles. Seedlings (<1.5 years old) had no measurable wood-grain angle and clockwise and counter-clockwise phyllotaxis occurred in equal proportions. However, young trees (13–15 years since planting) had a pronounced clockwise bias to their wood-grain spiral in contrast with a counter-clockwise bias in phyllotaxis. In contrast, mature trees (≥100 years old) had the reverse trend and exhibited a counter-clockwise bias in wood-grain spiral but a clockwise bias in phyllotaxis. A model is proposed to explain how chirality of spiral wood grain could generate an inverse bias in the chirality of phyllotaxis in lodgepole pine.

Key words: lodgepole pine, *Pinus contorta* var. *latifolia*, phyllotaxis, generative spiral, Fibonacci numbers, spiral wood grain.

Résumé : Jusqu’ici, on a jamais examiné la relation entre la chiralité de la spirale phyllotaxique et celle de la spirale du grain du bois, et encore moins la cause ou l’ontogénèse de ces relations. A cette fin, les auteurs ont comparé la chiralité de la spirale phyllotaxique des faisceaux foliaires et les écailles des cônes, avec la spirale des grains du bois chez des plantules, chez de jeunes arbres et chez des arbres adultes du pin lodgepole des Montagnes Rocheuses (*Pinus contorta* var. *latifolia*), au centre de la Colombie-Britannique. Pour évaluer la chiralité de la phyllotaxie, les auteurs ont déterminé la relation entre le nombre de parastiches contactées et la chiralité de la phyllotaxie des écailles des cônes et des faisceaux foliaires sur des rameaux. La présence de 3 ou 8 parastiches contactées dans le sens des aiguilles de la montre en direction acropète indique une spirale générative dans le sens des aiguilles de la montre, alors que des totaux de 2, 5 ou 13 parastiches acropètes indiquent une spirale générative dans le sens contraire des aiguilles de la montre. Les tiges du pin lodgepole sont presque toujours chimériques, à savoir qu’elles possèdent à la fois des phyllotaxies dans le sens et contre le sens des aiguilles de la montre sur le même individu, mais on retrouve généralement une forte correspondance entre la chiralité des phyllotaxies des écailles des cônes et celle des fascicules foliaires sous-jacents. Les plantules (<1.5 ans) ne montrent aucun angle du grain du bois mesurable et les phyllotaxies dans le sens et contre le sens des aiguilles de la montre surviennent en proportions égales. Cependant, les jeunes arbres (13-15 ans) montrent une spirale du grain du bois avec une forte tendance dans le sens des aiguilles de la montre, alors que la tendance est inverse au niveau de la phyllotaxie. Au contraire, les arbres adultes (100+ ans) montrent une tendance inverse alors qu’on observe un biais vers la spirale à contre sens des aiguilles de la montre au niveau du grain du bois, mais un biais vers le sens des aiguilles de la montre dans la phyllotaxie. Les auteurs proposent un modèle pour expliquer comment la chiralité de la spirale des grains du bois peut générer un biais inverse dans la chiralité de la phyllotaxie chez le pin lodgepole.

Mots clés : pin lodgepole, *Pinus contorta* var. *latifolia*, phyllotaxie, spirale générative, nombres de Fibonacci, spirale du grain du bois.

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Introduction

In pine (*Pinus* spp.), fascicles of needles on branches or cone scales on cones are initiated acropetally in a spiral (helix) termed the generative spiral, such that successive units are produced at or near the "golden angle" (~137.5°) apart. This geometric symmetry in plants such as pine can be derived from proportions based on the Fibonacci (F) number series (0, 1, 1, 2, 3, 5, 8, 13, …) (Adler et al. 1997). One can easily find these F numbers in pine. For example, the number of diagonal files of cone scales (parastichies) ascending in clockwise (CW) and counter-clockwise (CCW) directions in any circumference of a cone sum to consecutive F numbers in the series. The generative spiral of a plant is either CW or CCW after germination and does not appear to be under genetic control, i.e., no plant species has been found to deviate from the expected frequency of 50% CW : 50% CCW regardless of seed source (Bachmann 1983). However, no studies were found that clearly examined whether or how phyllotactic direction (chirality) changes over time in a long-lived plant, such as pine.

Perhaps the only case where the generative spiral direction in mature trees has been studied in a rigorous manner, although not ontogenetically, has been in palms (e.g., Davis 1970). Davis (1974) showed that the direction of the generative spiral in palm was strongly correlated with geographic latitude, with a majority of palms in the Northern Hemisphere exhibiting a CW spiral relative to a predominance of CCW forms in the Southern Hemisphere. In a reanalysis of Davis' data, Minorsky (1998) demonstrated that the earth's magnetic fields may play a more substantial role than latitude in influencing spiral direction in palm. However, irrespective of the causative agent, it provides a clear example of the additional complexity associated with spiral direction in long-lived plants. Several questions cannot readily be addressed with this system. First, palms generally have a single apical shoot meristem and no secondary stem growth. Thus, one cannot evaluate whether the original generative spiral direction present in the seedling is propagated in lateral, dormant, and reproductive meristems. Second, Davis' analysis did not take tree age into account. Thus, it is not evident whether young palm germinants were affected, providing what would then be the first example of a spiral direction bias in seedlings, or whether selective mortality and (or) interconversion between spiral directions is occurring with palm tree age.

Pine species present a useful system with which to address the questions raised here. First, the generative spirals in cone scales and needle fascicles result in a geometry that is predicted by the F number series (see above). Second, the architecture and overwintering meristematic buds in mature pine provides a suitably complex system with which to address the issue of how a generative spiral is propagated through the adult tree form. Third, it is one of the most abundant, commercially important, and most highly planted tree species in British Columbia, yet little if anything is known about its demographics with respect to spiral handedness. Lodgepole pine (*Pinus contorta* Dougl. ex Loud.) is named for a spiral of a different type, the spiral of the wood grain. This latter property is of commercial interest, because excessive wood-grain spiral is an undesirable trait. Finally, the vascular primordia in pine meristems, called sympodia, also occur in F numbers providing suggestive evidence that vascular organization and phyllotaxis in pine may be interrelated (Namboodiri and Beck 1968).

The linkage between the organization of vascular tissue and phyllotaxis has been considered likely as early as the middle of the 19th century (for a review, see Esau 1965). For example, leaf vascular traces precede leaf primordia, which they later become associated with in a variety of trees including conifers (Sterling 1945; Gunckel and Wetmore 1946). Furthermore, the number of sympodia in conifers with helical phyllotaxis, along with limiting angle and direction of leaf trace divergence, can be used to predict the generative spiral direction and vice versa (Namboodiri and Beck 1968). However, what has not been integrated into the larger picture is whether spiral wood grain, in particular its chirality, can in any way influence the chirality of the phyllotactic spiral. Pine again provides an interesting system with which to study this question. Northcott (1957) showed that wood-grain spiral in individuals of lodgepole pine change with age in a highly predictable fashion, beginning with CW acroptetal spiral in young trees and shifting to a CCW spiral in mature trees. The possibility of a linkage between spiral of wood grain and phyllotaxis has not been explored.

The objectives for this study were (*i*) to examine the inter-relationships between the Fibonacci-based spirals in cone-scale and needle-fascicle phyllotaxis, (*ii*) to characterize the chronology and frequency of spiral direction from seedling to maturity, and (*iii*) to ascertain if any relationship exists between the direction of spiral in wood grain and the direction of the generative spiral in cone scales and needle fascicles in Rocky Mountain lodgepole pine (*P. contorta* var. *latifolia* Engelm.).

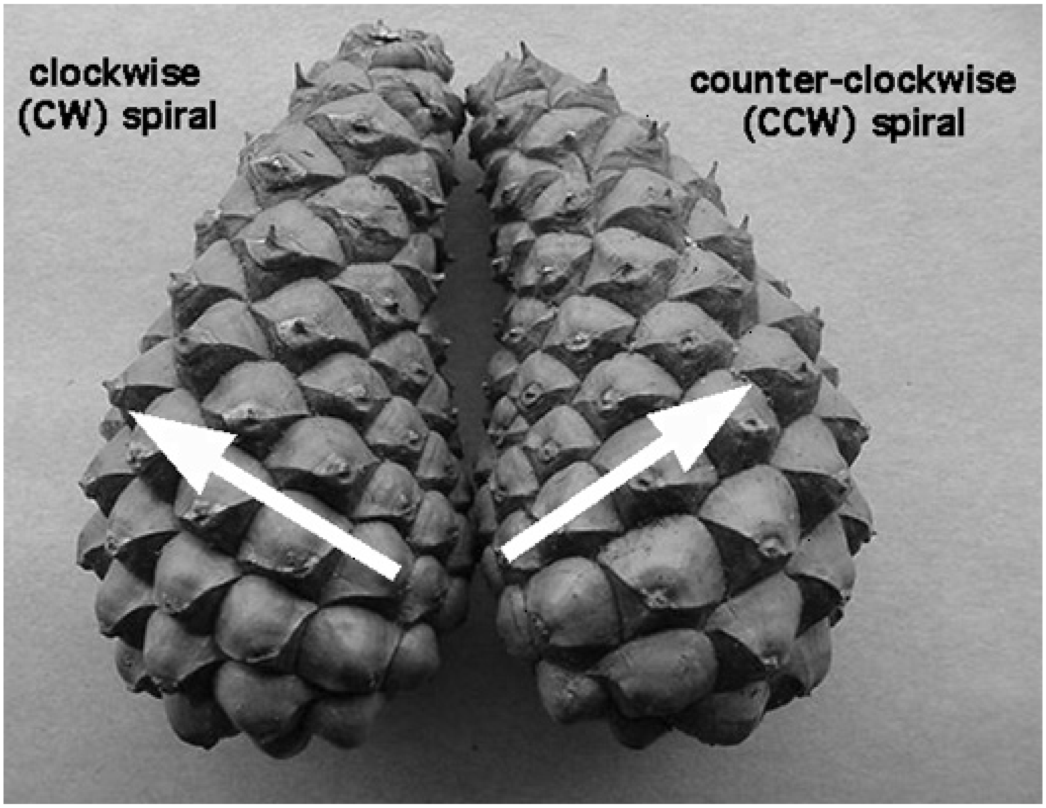
Materials and methods

Phyllotaxis, parastichies, and generative spiral in lodgepole pine

The spiral phyllotaxis of needle fascicles on branches and cone scales on cones in Rocky Mountain lodgepole pine creates contact parastichies (readily apparent diagonal files of plant parts) in both directions, which sum to consecutive F numbers (2, 3, 5, 8, 13…) over a complete circumference of the branch or cone, respectively. The chirality of parastichies in lodgepole pine needle fascicles or cone scales was either CW or CCW) (Fig. 1). Since it was far easier to count parastichies than to determine the direction of the generative spiral (the spiral pattern in which new primordia are initiated in the meristem), we determined the relationship between parastichies and generative spiral and report only the direction of the acropetal (ascending towards the apex) generative spiral in this paper.

Needle-fascicle phyllotaxis was occasionally («1% frequency) difficult to categorize as either CW or CCW. These rare branchlets typically had CW:CCW or CCW:CW parastichies of [2:4] or [4:6]. Deviation of cone-scale parastichies from expected F numbers was also extremely rare. In all cases, the rare cone or branch samples with phyllotaxis not conforming to the F number series were simply excluded from the analysis.

Fig. 1. Lodgepole pine cones with clockwise (CW) and counter-clockwise (CCW) acropetal generative spirals. Chirality of the generative spiral is the same as the direction of the contact parastichies that sum to 8 in a complete circumference (white arrows).



Phyllotaxis in lodgepole pine seedlings

The phyllotaxis of fascicles of needles on branches was assessed in seedlings (<1.5 years old after seeding) grown in a greenhouse in peat substrate and styroblocks at the J.D. Little Nursery (Northwood Trust, CANFOR, Prince George, B.C., Canada). Three separate "batches" of seedlings were assessed, where each batch was comprised of five seedlots collected throughout the interior of British Columbia, utilizing a sample size of approximately 100 (94–104) seedlings per seedlot. Heights of seedlings in batches 1, 2, and 3 ranged from 5–10, 15–25, and 20–30 cm, respectively, as a result of late to early seeding dates. Seedling shoots were predominantly apical leaders with no or negligible lateral branching. As a result, seedling phyllotaxis was based solely on the needle-fascicle phyllotaxis of the leader.

Young lodgepole pine plantations

Young trees (13–15 years since planting) were randomly selected at 10-m intervals along transects 10 m apart in three separate commercial plantations in central British Columbia in summer 1999. The first plantation (planted 1986) was located 0.5 km west of the University of Northern British Columbia (UNBC), Prince George, B.C. (54°05′N, 122°50′W); the second (planted 1985) was approximately 30 km east of New Hazelton, B.C. (54°14′N, 121°26′W); and the third (planted 1984) was near Westlake, B.C. (54°15′N, 122°58′W). Trees were assessed for direction of needle-fascicle or cone-scale phyllotaxis in the main stem and in 30 random primary (1°) branches, defined as those arising from the main stem. In all cases, needle-fascicle phyllotaxis was assessed on branch segments that subtended cones.

Direction of wood-grain angle was also assessed in the UNBC plantation trees to determine if a relationship existed between direction of wood-grain spiral and direction of needle-fascicle or cone-scale phyllotaxis in young trees. Wood-grain angle was determined by removing the bark of the young tree at three locations along the main stem axis (at approximately 0.6, 1.2, and 2 m above ground level) and measuring wood-grain angle using a protractor with the 0° axis oriented along the vertical axis of the tree.

Mature lodgepole pine

A natural sub-boreal forest dominated by lodgepole pine, located 52 km southeast of Prince George, B.C. (53°20′N, 123°38′W), was logged in August and September of 1999. According to silvicultural records taken prior to harvesting, the mean age of pine in the stand was 127 years. Trees were piled with branches intact as they were felled, providing easy access to tree crowns for cone and branch sampling. One hundred trees were randomly selected from those felled from along the margins of approximately 2 km of logging road within the cutblock. A total of 30 branch samples (including cones if present on the branches sampled) were collected from random canopy positions within each tree crown into single large paper bags and transported back to the laboratory for analysis. Direction of the generative spiral was determined for all cones as well as fascicles on branchlets contained on the branch samples collected. It was impossible for safety as well as accessibility reasons to assess wood-grain angle on the same trees sampled for phyllotaxis.

In most cases, cones were primarily attached to the primary axis of the branch sample, hereafter referred to as the 1°

branch, with fewer numbers of cones on branchlets arising from the 1° branch, hereafter referred to as secondary 2° branch. Primary branches typically had stem diameters <3 cm in diameter. Larger branches were mostly needleless with fascicle scars that were difficult to discern and, therefore, were not scored. Chirality of needle-fascicle phyllotaxis subtending cones was always recorded to permit the assessment of correspondence between branchlet and cone chirality.

In a separate survey, cones were collected from beneath mature trees in natural sub-boreal forest in a 3-km radius of the UNBC campus, Prince George, B.C. Cones were assessed as to direction of the generative spiral. Care was taken to sample no more than 20 cones/tree for each of >100 trees sampled.

Relationship between tree age and wood-grain angle

We sought to determine if a relationship existed between tree age and wood-grain angle in lodgepole pine. To this end, 100 mature logs were selected at random from those delivered to the Lakeland Lumber Mill, Prince George, in summer 2000. Trees were harvested from eight different cutblocks from two different sites (54°25′N, 123°14′W and 54°14′N, 123°00′W, respectively) approximately 50 km north-northeast of Prince George.

Logs were laid out horizontally in the mill yard and strips of bark were removed from lower, middle, and upper portions of logs to reveal “windows” on the most recent annual increment. Pine logs averaged 15 m in length and windows in the bark averaged 43 cm in length and 10 cm in width, 0.42, 7.1, and 13.5 m from the log base, respectively. To accentuate the wood grain, 20–30 black drafting pins were inserted in the grain along the length of the exposed window and digital pictures taken using a digital camera (Mavica MVC-FD88, Sony Corp.) at a resolution of 1.3×10^6 pixels per image. Images were edited to size and converted to black and white using image processing software (Adobe Photoshop version 5.0.2, Adobe Systems Inc., U.S.A.) and printed on paper at 1200 dots/in. (1 in. = 2.54 cm) with a laser printer. Wood-grain angle was determined on the printed copies of the wood-grain images using a drafting table with parallel rule, set square, and protractor. Finally, a 6 cm deep “cookie” was sawn off the base of each log for determination of tree age. Cookies were sanded and growth rings counted using a flat-bed scanner and tree-ring analyzing software (WinDENDRO, Regent Instruments, Canada). Three of the 100 cookies could not be aged because of heart rot in the tree stem.

Statistical analyses

The χ^2 test was used for all null-hypothesis testing. Linear regression equations were generated using a graphics software package (Deltagraph, version 4.5; SPSS Inc., Chicago, Ill.).

Results and discussion

The number of cone-scale or needle-fascicle contact parastichies in a conifer cone or branch circumference has long been noted to abide by the F number series, with Schimper (ca. 1830s; see Jean 1984) perhaps being the first. Specifically, the number of parastichy spirals in one direction versus that in the opposite direction in Rocky Mountain lodgepole pine were found to sum to consecutive F numbers, i.e., 8:13 versus 13:8 in cones and 2:3 versus 3:2 or 3:5 versus 5:3 in needle fascicles. While the frequencies of left-handed (CW) or right-handed (CCW) cones have variously been reported for a variety of pines, we present here a more thorough characterization of the chirality (or handedness) of phyllotaxis and wood grain in lodgepole pine.

We first ascertained the relationship between the F number of CW versus CCW contact parastichies in cone scales and needle fascicles (which can be determined with relative ease), with the more developmentally relevant, but more difficult to determine, chirality of generative spiral. We report here that 3 or 8 CW acropetal parastichies (nonconsecutive F numbers) indicate an acropetal CW generative spiral, while totals of 2, 5 or 13 CW (or 3 or 8 CCW) acropetal parastichies indicate a CCW generative spiral. Lodgepole pine cones with 8 CW and 8 CCW acropetal parastichies are shown in Fig. 1, the former indicating a CW generative spiral and the latter a CCW generative spiral. The same relationship between contact parastichies and generative spiral also held true for vegetative structures, namely the arrangement of needle fascicles on branches (not shown). The only other explicit statement of this relationship was for flax, *Linum usitatissimum* (Girolami 1953).

We first examined seedlings to determine what the frequency of CW:CCW pine were within 1.5 years of germination. The prediction, based on observations for a large range of plant species (but mostly short-lived nonwoody species) was that there would be equal numbers of CW versus CCW pines, i.e., a frequency of 50% CW : 50% CCW (Bachmann 1983). Among the seedlings surveyed, the actual numbers of seedlings with CW versus CCW phyllotaxis did not deviate significantly from the expected frequency of 50:50 (Table 1). This result held true for each of the nine seedlots analyzed independently as well as for the combined data from all seedlots.

Less well documented in the literature is the persistence of the initial generative spiral direction over time, especially in woody perennials. As a result, we set out to characterize the chirality of phyllotaxis, and also wood grain, in a number of lodgepole pine populations of different ages across central British Columbia.

Phyllotaxis in young and mature lodgepole pine trees

While young seedlings with a single leader were necessarily either CW or CCW, a single statement of phyllotaxis in young and mature trees was less straightforward. Specifically, young to mature trees nearly always contained a mixture of CW and CCW needle-fascicle and cone-scale phyllotaxies such that trees ranged from 0 to 100% of a given spiral direction or chirality at the tree level. Thus, a simple statement of chirality (see Kihara 1972) in lodgepole pine was not possible. Therefore, we report chirality of tree populations in two ways: (*i*) meristem: where meristems (cone, needle, or cone + needle) from a population of trees were pooled or (*ii*) tree: where meristems were grouped by individual tree (unpooled) and individuals scored as being either CW or CCW, even though trees were nearly always chimeric in their phylotactic spiral directions.

Among young lodgepole pine (13–15 years since planting) from three different plantations, all tree or meristem measures of chirality showed a CCW bias (Table 2). Spe-

Table 1. Number and percentage of lodgepole pine seedlings (<1.5 years old) with a clockwise (CW) generative spiral.

Batch	Seedlot	*n*	CW(no.)	CW(%)	χ^2	*p* value ($\nu = 1$)
1	32274	104	50	48	0.154	ns*
	32275	104	50	48	0.154	ns
	28762	104	47	45	0.962	ns
	60120	104	43	41	3.115	ns
	30809	104	50	48	0.154	ns
2	31381	104	56	54	0.615	ns
	35185	94	48	52	0.043	ns
	32775	100	55	55	1.000	ns
	30908	100	52	52	0.160	ns
	31389	100	43	43	1.960	ns
3	32706	99	52	52	0.252	ns
	31394	100	49	49	0.040	ns
	41910	100	48	48	0.160	ns
	28762	100	57	57	1.960	ns
	28762	100	52	53	0.160	ns
Total	—	1517	752	51.7	0.114	ns

*ns, not significant ($p > 0.05$).

Table 2. Number and percentage of needle fascicles and (or) cone scales exhibiting clockwise (CW) acropetal phyllotaxis in 13- to 15-year-old lodgepole pine trees in three plantations in central British Columbia.

Phyllotaxy	Plantation	*n*	CW(no.)	CW(%)	χ^2	*p* value ($\nu = 1$)
Pooled samples						
Needles	Total	3327	1608	48.33	3.7	0.1
Cones	Total	1885	900	47.75	3.83	0.1
Needles + cones	Total	5212	2508	48.12	7.37	0.005
Unpooled samples						
Needles	FFTW	76	36	47.4	0.21	ns*
	WestLake	37	16	43.2	0.68	ns
	Hazleton	34	14	41.2	1.06	ns
	Total	147	66	44.9	1.53	ns
Cones	FFTW	51	20	39.2	2.37	ns
	WestLake	26	11	42.3	0.62	ns
	Hazleton	27	10	37.0	1.81	ns
	Total	104	41	39.4	4.65	0.05
Needles + cones	FFTW	74	32	43.2	1.35	ns
	WestLake	37	16	43.2	0.68	ns
	Hazleton	34	15	44.1	0.47	ns
	Total	145	63	43.4	2.49	ns

Note: Phyllotaxis samples were either pooled across all trees (meristem chirality), or left unpooled such that each tree was scored as being either predominantly CW or CCW (tree chirality). Plantations were either considered separately or collectively (total). A significant *p* value resulting from the chi-square (χ^2) test indicates deviation from the null hypothesis that phyllotaxis should be CW in 50% of the samples.

*ns, not significant ($p > 0.1$).

cifically, tree chirality (unpooled samples) ranged from 37 to 47% CW, while meristem chirality (pooled samples for all three populations) ranged from ~47.8 to 48.3% CW for needle, cone, and (needle + cone) samples. On a meristem basis, a prevailing CCW spiral was nearly significant in pooled needle or cone samples (χ^2, $p < 0.1$) but highly significant (χ^2, $p < 0.005$) in pooled (needle + cone) samples. Only cone (39% CW) chirality was significant (χ^2; $p < 0.05$) at the tree level (unpooled samples).

Chirality was reversed in mature trees relative to young trees (Table 3). Specifically, the generative spiral in mature trees was uniformly CW, ranging from 51.1 to 51.9% CW in

Table 3. Number and percentage of needle fascicles and (or) cone scales exhibiting clockwise (CW) acropetal phyllotaxis in mature lodgepole pine trees (n = 100) from a natural forest stand in central British Columbia.

Phyllotaxis	Sample size	CW(no.)	CW(%)	χ^2	p value (ν = 1)
Pooled samples					
1° needles	2 834	1468	51.8	3.67	0.1
1° cones	5 546	2840	51.21	3.24	0.1
1° and 2° needles	5 182	2691	51.93	7.72	0.01
1° and 2° cones	5 681	2902	51.08	2.66	ns*
1° and 2° needles + cones	10 863	5593	51.49	9.6	0.005
Unpooled samples					
1° needles	99	52	52.5	0.25	ns
1° cones	97	61	62.9	6.44	0.025
1° and 2° needles	96	52	54.2	0.67	ns
1° and 2° cones	97	63	65.0	8.67	0.005
1° and 2° needles + cones	95	55	57.8	1.44	ns

Note: Cones were found on primary (1°) and secondary (2°) branches (see Materials and methods for more detail). Phyllotaxis samples were either "pooled" across all trees (meristem chirality), or left "unpooled" such that each tree was scored as being either predominantly CW or CCW (tree chirality). A significant p value resulting from the chi-square (χ^2) test indicates deviation from the null hypothesis that phyllotaxis should be CW in 50% of the samples.

*ns, not significant (p > 0.1).

Table 4. The degree of correspondence between the direction of the acropetal generative spiral in cone scales in cones and that of the needle fascicles on the branch subtending the cone in 13- to 15-year-old lodgepole pine trees from three plantations in central British Columbia.

Plantation	Sample size	No. the same	Similarity (%)	χ^2	p value (ν = 1)
FFTW	912	817	89.6	572	0.001
West Lake	400	339	84.8	193	0.001
Hazelton	394	317	80.4	146	0.001
Total	1706	1473	86.3	901	0.001

Note: The number and percent of times when cone and needle spiral direction were the same is indicated. Significant p values for the chi-square (χ^2) analysis indicate deviation from the null hypothesis that subtending branch needle spiral direction would have no bearing on cone spiral direction, i.e., spiral direction in branch and cone would match 50% of the time.

Table 5. The degree of correspondence between the chirality of phyllotaxis in needle-fascicles on 2° versus subtending 1° branch (n–n), or between cone scales in cones and that of needle fascicles on subtending branches (c–n) from 100 mature lodgepole pine trees in a natural forest stand in central British Columbia.

Phyllotaxis	Sample size	No. the same	Similarity (%)	χ^2	p value (ν = 1)
n–n	2300	1376	59.8	88.8	0.001
c–n (1°)	5470	3675	67.2	646	0.001
c–n (2°)	338	105	31.1	48.5	0.001
c–n (1° and 2°)	5808	3780	65.1	528	0.001

Note: The number and percent of times when phyllotaxis spiral direction were the same is indicated. Significant p values for the chi-square (χ^2) analysis indicate deviation from the null hyppothesis that phyllotactic spiral direction would have no bearing on future spiral direction, i.e., spiral directions would only match 50% of the time. Primary (1°) and secondary (2°) branches are defined in Table 3.

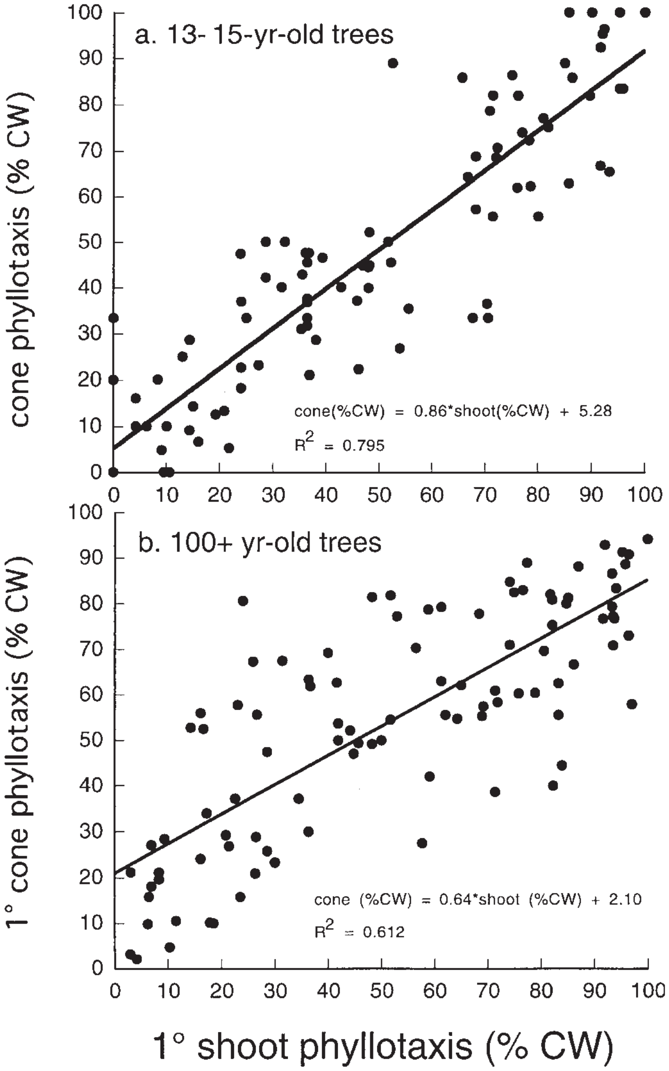
pooled meristem samples, and 52.5 to 65% at the tree level. As in young trees, chirality was only significant at the tree level when considering cones, i.e., in 1° cones (χ^2, p < 0.025) and also in combined 1° + 2° cones (χ^2, p < 0.005). After pooling samples (meristem level), a prevailing CW spiral was nearly significant in pooled 1° branches and 1° cones (χ^2, p < 0.1) and significant in combined 1° + 2° branch meristems (χ^2, p < 0.01).

To further confirm a CW bias to phyllotaxis in mature trees, cones were gathered from naturally growing mature lodgepole pine trees in a 3-km radius of the UNBC campus. Of the 2053 cones collected, a significant majority (1090; 53.1% of the total) had a CW generative spiral (χ^2; p < 0.01).

Correspondence between needle and cone phyllotaxis

While young and mature trees were generally chimeric in meristem spiral chirality, there was a highly significant correspondence between cone spiral direction and that of the needle-fascicle phyllotaxis of the subtending branch (Tables 4 and 5). To assess the significance of the propagation of chirality from vegetative to reproductive meristems, we tested our data against the null hypothesis that each cone meristem would arbitrarily develop in a CW or CCW fashion, similar to that seen for populations of young seedlings (e.g., Bachmann 1983; Table 1). In both young and mature trees, cone spiral direction matched subtending branch needle phyllotaxis direction in 86 (Table 4) versus 65% (Table 5) of the cases (χ^2; p < 0.001), respectively.

Fig. 2. Correspondence between the percentage of a tree's cone-scale versus subtending needle-fascicle phyllotaxis that is clockwise (CW) in (*a*) 13- to 15-year-old lodgepole pine ($n = 93$) versus (*b*) ≥100-year-old mature lodgepole pine ($n = 98$). Linear regression equations and correlation coefficients are given.



Thus, chirality of phyllotaxis was propagated from vegetative meristem to reproductive meristem with some degree of fidelity. This relationship was also verified by plotting the percentage of CW reproductive versus vegetative phyllotaxies at the tree level. Correlation coefficients of 0.795 (Fig. 2*a*) and 0.612 (Fig. 2*b*) were observed in young and mature populations, respectively. Thus, at least in a statistical sense, chirality of phyllotaxis was propagated from branch to cone. In addition, a sufficient number of 2° branches were obtained permitting us to determine whether chirality of the 1° branch was propagated in derivative (e.g., 2°) branches (Table 5, n–n). Our data show that chirality of vegetative phyllotaxis is indeed propagated from 1° to 2° branches (χ^2; $p < 0.001$: Table 5).

Zagórska-Marek (1985) examined phyllotactic transitions along axes of branches in 1- to 12-year-old *Abies balsamea* (L.) Mill. and observed 92% fidelity in propogation of chirality between sequential years of vegetative growth. This corresponds reasonably well with the 86% correspondence between cone-scale and needle-fascicle phyllotactic direction seen for lodgepole pine in this study (Table 4).

In mature trees, there was added complexity in that cones occurred on 1° (main axis of the branch sample) as well as 2° branches (next order of branch off of 1° branches). While chirality of cones on 1° branches showed a high degree of correspondence with that of subtending branches, cones on 2° branches (representing <6% of the total number of cones), exhibited a negative correspondence (in 69% of the samples) with subtending needle-fascicle spiral direction (χ^2; $p < 0.001$; Table 5). One explanation for this reversal of concordance between cone-scale and subtending needle-fascicle phyllotactic direction of 2° versus 1° branches can

Fig. 3. The relationship between age of lodgepole pine trees and the mean acropetal wood-grain angle of the tree stem. A clockwise acropetal wood-grain angle is one in which the wood-grain ascends to the left of vertical. A third-order polynomial equation was fit to the data and forced through 0 on the *y* axis to account for the fact that 1-year-old pine seedlings did not have a wood-grain angle: $f(x) = -2.89 \times 10^{-5} x^3 + 7.22 \times 10^{-3} x^2 - 0.418x$. Data from Northcott (1957) for spiral wood grain in lodgepole pine ($n = 37$) are shown for contrast.

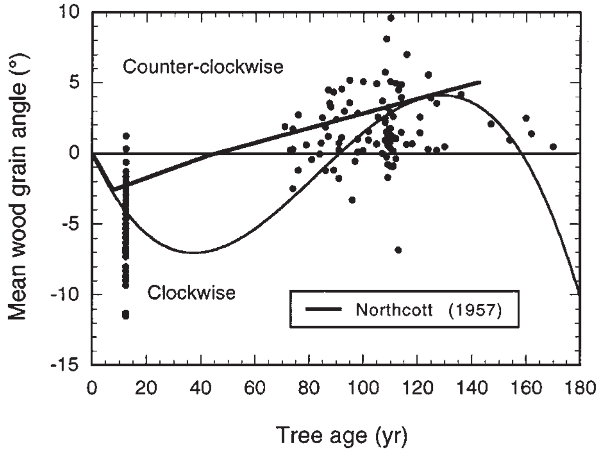


Fig. 4. Chirality of ascending wood grain (wood-grain angle) and phyllotaxis (% CW) versus tree age in lodgepole pine.

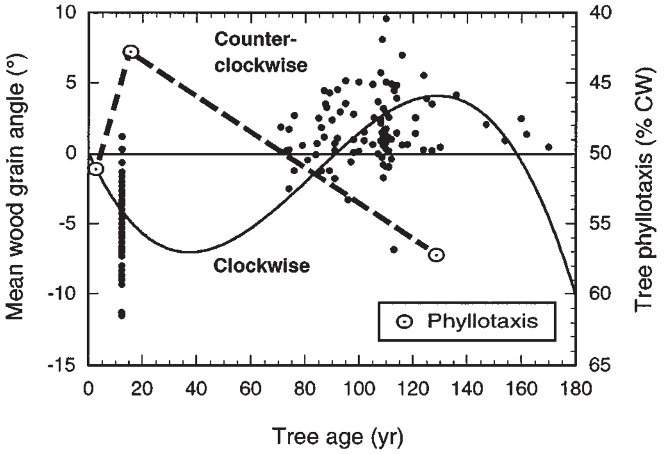


Table 6. Relationship between number of sympodia and the concordance between the direction of generative spiral and leaf trace divergence in pine (adapted from Table 3 in Namboodiri and Beck 1968).

No. of sympodia	Relationship
5, 13	Concordant
3, 8, 21	Discordant

Note: Because the divergence angle for *Pinus contorta* is 137.5°, the table has one less variable than that of Namboodiri and Beck. A simplified version for 13 sympodia is shown in Fig. 5*a*.

be derived from the results of Namboodiri and Beck (1968) and the model that we propose later (see Discussion below).

Wood-grain spiral direction in lodgepole pine

In 13-year-old pine (UNBC plantation: n = 75), 97% of individuals had a CW wood-grain spiral with a mean angle of –5.19 ± 2.46° (n = 3/tree) (Fig. 3). In contrast, 82% of mature trees (n = 100), with a mean age of 106 years, had a CCW wood-grain spiral (n = 3/tree) with a mean angle of +1.73 ± 2.48°. These data strongly suggest that chirality of acropetal wood-grain spiral (beginning with no bias in seedlings), shifts from being CW in young trees to CCW in mature trees (Fig. 3). This trend was previously observed by Northcott (1957, Fig. 3) and is confirmed here using more northerly populations of lodgepole pine and a different methodology; Northcott sampled fewer trees (n = 37) but developed an age versus wood-grain direction profile by sequentially removing annual increments and determining wood-grain angle for each. Most Northern Hemisphere conifers exhibit a shift in wood-grain angle direction with tree age, most of these from CW to CCW acropetal spiral as is the case with lodgepole pine (see Harris 1989; Kubler 1991). However, the possibility that wood-grain angle could influence other aspects of tree form, such as phyllotactic spiral direction, has to our knowledge not been observed or conjectured previously.

Inverse relationship between chirality of wood grain and the bias in phyllotactic spiral direction

We have shown that the chiralities of acropetal wood grain and phyllotaxis are inversely related in lodgepole pine. More precisely, our data show that juvenile lodgepole pine (10–15 years old) have CW wood-grain spiral but a bias towards CCW phyllotaxis, while mature pine (≥100 yearsold) have a predominantly CCW wood-grain spiral but a bias towards CW phyllotaxis (Fig. 4). While this inverse relationship did not conform to our initial hypothesis, i.e., that wood-grain angle, if related to phyllotaxis, would bias it in the direction of the predominant wood-grain angle, there were a number of additional reasons to examine the relationship more carefully.

First, the literature does provide some empirical (e.g., Esau 1965) and theoretical (e.g., Jean 1984) support for a linkage between vascular organization at the meristem level and phyllotactic spiral direction (see also Steeves and Sussex 1989). In particular, leaf vascular traces appear to precede initiation of leaf primordia in a variety of trees including many conifer species (Sterling 1945; Gunckel and Wetmore 1946). Second, the number of vascular primordia (sympodia) in pine (which occur in F numbers) and the direction of divergence of the needle-fascicle vascular traces from them, is predicted by the phyllotactic spiral direction and vice versa (Namboodiri and Beck 1968). Specifically, they showed that meristems of conifers such as pine with 3, 8, or 21 sympodia would have a generative spiral direction discordant with direction of leaf trace divergence, while those with 5 or 13 sympodia would have a generative spiral concordant with leaf trace divergence (Table 6). This last observation, in particular, is key to producing a tenable mechanism to explain our results.

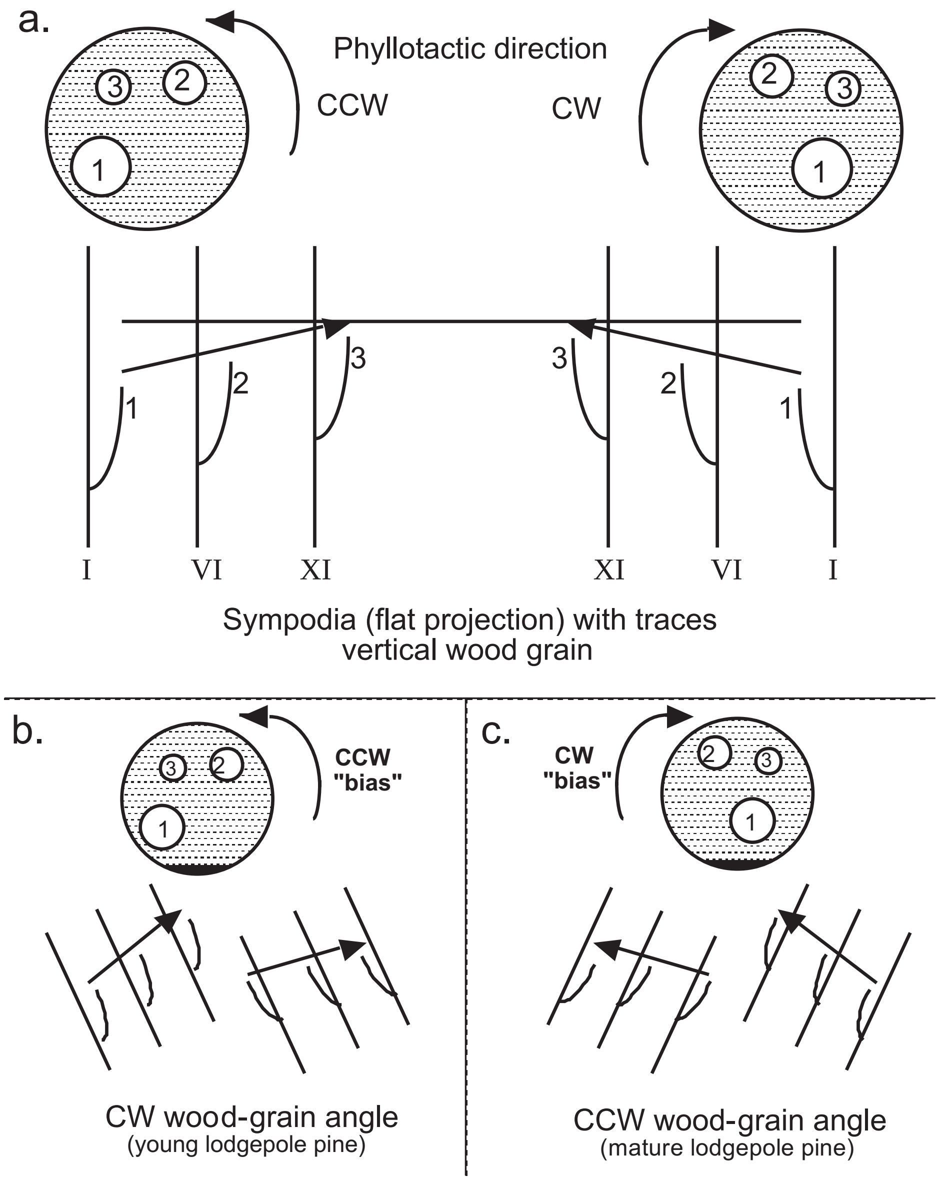
A mechanism to explain the opposing bias in chirality of wood-grain and phyllotactic spiral

It is still an open question as to which process (i.e., primordial initiation or procambial strand development) might precede and thus potentially dictate the pattern in the other in the developing meristem. Nevertheless, we are compelled by the detailed studies of spiral phyllotaxis and vascularization of meristems in poplar by Larson (e.g., 1975, 1977) as well as the findings of Namboodiri and Beck (1968) and our own data for lodgepole pine, to propose a model consistent with the "procambial strand hypothesis" (Larson 1983). As such, our model purports that procambial leaf traces precede (e.g., Gunckel and Whetmore 1946) and dictate the arrangement of primordia on the meristem, most likely via an acropetally propagated chemical or mechanical pressure or strain stimulus (e.g., see Selker et al. 1992). Such a model would be consistent with the way in which chirality of wood-grain spiral, which changed from CCW to CW in young to mature trees of lodgepole pine, was inversely related to the pattern of bias in chirality of phyllotaxis in pine (Fig. 4).

Our model requires that there be a correspondence between the chirality of the macroscopic wood-grain angle of the main stem (secondary vascular tissue) with that of the branches and, finally, with that of the sympodia and the precocious procambial strands, which precede them in the meristem itself. While we, and to our knowledge others, have not yet demonstrated definitively that such a correspondence exists, published work does support this idea. For example, spiral wood grain in the main stem of trees is propagated in branches along annual growth increments of the same age (Harris 1989; Kubler 1991) and between sequential annual increments (Brazier 1965). It is also true that wood-grain orientation is propagated axially through successive years of growth, established by the orientation of cambial initials that precede tracheid production (Harris 1989). Finally, since studies by Larson (1980) have demonstrated that the primary vascular system organization serves as both the structural and organizational template for secondary vascularization, we infer that wood-grain spiral is a true reflection of spiral in the procambial strands within the meristem. However, it is clear that further study will be required to confirm the correspondence between chirality of wood-grain spiral and that of procambial strands.

We simplify the pictorial representation of our mechanism by considering, at least initially, only the most com-

Fig. 5. A diagram explaining how wood-grain angle could influence the chirality of phyllotaxis in pine based in part on the work of Namboodiri and Beck (1968). In pine, meristems have a Fibonacci number of sympodia. When these sympodia number 5 or 13, leaf traces diverge in the same acropetal direction as the generative spiral, and in the opposite direction when sympodia number 8 or 21. Meristems in lodgepole pine commonly have 13 sympodia, numbered I to XIII, with leaf traces diverging from every fifth sympodia. (*a*) In vertical-grained pine, CCW divergence leads to CCW phyllotaxis, and CW divergence leads to CW phyllotaxis. (*b*) However, when a CW wood grain occurs, as in young lodgepole pine, a CCW "bias" in phyllotaxis results. (*c*) Conversely, when a CCW wood grain occurs, as in mature lodgepole pine, a CW "bias" in phyllotaxis results.



mon number of sympodia found in lodgepole pine meristems, which we have found to be 13 (A.L. Fredeen, J.A. Hoekstra, and R.W. Madill, unpublished data). For simplicity, the "ring" of sympodia are projected into a plane (viewed from the outside) with only those three sympodia (I, VI, and XI) associated with the first three primordia (oldest to youngest) shown (Fig. 5), since a third primordium establishes the chirality of phyllotaxis. In the absence of a wood-grain spiral, e.g., in seedlings (Fig. 5*a*), sympodia are aligned parallel to the branch axis, and a CCW divergence of leaf traces results in a CCW phyllotactic generative spiral and vice versa. This particular case would be the ideal case conforming to observations of Namboodiri and Beck (1968; Table 6). However, in the case of young lodgepole pine, a CW wood-grain spiral would counteract CW phyllotaxis, thereby introducing a CCW "bias" to the phyllotaxis (Fig. 5*b*). In contrast, the CCW wood-grain spiral found in mature lodgepole pine would have the opposite effect, thereby enhancing the CW bias to phyllotaxis (Fig. 5*c*). Thus, it can be seen that as long as the sympodia number 13 (or 5), wood-grain spiral direction would bias the phyllotaxis in the opposite direction. While this result seems initially to be counterintuitive, it is an obvious consequence of the geometry when viewed pictorially in Fig. 5. One obvious outcome of the model as proposed is that the inverse bias in phyllotaxis should increase as wood-grain angle is increased.

Finally, we return to our observation that chiralities of phyllotaxis in cones versus subtending needle fascicles were positively related in 1° branches but negatively related in 2° branches (see Table 5). We believe that our proposed mechanism could also help to explain this puzzling result. Specifically, if sympodia numbered 13 (the common number in pine) in both cones and needle fascicles of 1° branches, then it would not be surprising if direction of meristem spiral was preserved from branch to cone. However, if sympodial number were to drop from 13 in 2° branches to 8 in cones on 2° branches, then the phyllotactic spiral direction would reverse from branch to cone (Table 6). In fact, the number of sympodia is seen to decrease in smaller diameter branches of conifer (Namboodiri and Beck 1968) and in lodgepole pine (A.L. Fredeen, J.A. Hoekstra, and R.W. Madill, unpublished data). However, further work will be required to establish how sympodial numbers are propagated from vegetative to reproductive meristems in pine.

Summary

Lodgepole pine, like other *Pinus* spp., has a spiral growth pattern that largely conforms to proportions found in the Fibonacci number series. We found that 3 or 8 CW acropetal parastichies indicate an acropetal CW generative spiral, while totals of 2, 5, or 13 CW acropetal parastichies indicate a CCW generative spiral in cone scale or needle phyllotaxis. Seedlings of lodgepole pine (<1.5 years) had no measurable wood-grain spiral and CW and CCW seedlings occurred in essentially equal proportions. However, young lodgepole pine trees (13–15 years since planting) had a pronounced CW bias to their wood-grain spiral in contrast to a CCW bias in their phyllotaxis. Mature lodgepole pine (≥100 years) had the reverse trend and exhibited a CCW spiral in their wood grain but a CW bias to their phyllotaxis. Thus, chirality of wood-grain appears to be inversely related to chirality of phyllotaxis. A mechanism is proposed to explain the inverse relationship, but verification of such a mechanism will require additional study.

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