

NOTE / NOTE

Primary longitudinal resin canals in lodgepole pine occur in Fibonacci numbers

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Abstract: Primary (nontraumatic) longitudinal resin canals (RCs) in immature (expanding) and mature stems of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) were found to occur in Fibonacci (F) numbers (specifically 5, 8, 13, and 21). The most commonly observed number of RCs in immature stem circumferences was 13 (53% of all immature stems surveyed), but 8, 21, and 5 were also observed in decreasing order of prevalence, respectively. In general, the greater the immature stem diameter, the higher the F number of the RCs. However, branch order appeared to have the greatest effect on the number of RCs. Specifically, the leader (1° axis) generally had 21 RCs in the terminal bud and 13 in the lateral buds. All other terminal axes (i.e., 2°, 3°, 4°) tended to 13, while associated lateral axes tended to 8. The same general relationships between number of RCs, stem diameter, and branch order were also observed for mature (woody) branchlets of lodgepole pine, for example, 13 RCs were also the most prevalent F number observed (i.e., in 48% of the observed branchlets). The phyllotactic chirality was also assessed for mature branch samples. A tree tends to retain a phyllotactic chirality (or handedness) as branch order increases, despite decreases in the number of RCs from 21 to 5 from low to high branch orders. The implication of these findings to helical phyllotactic development in conifers is discussed.

Key words: Fibonacci numbers, resin canals, lodgepole pine, *Pinus contorta* var. *latifolia*, phyllotaxis.

Résumé : Les auteurs ont observé que les canaux résinifères (RC) longitudinaux primaires (non-traumatiques), dans les tiges en croissance (en expansion) et matures du pin lodgepole (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.), se retrouvent selon les nombres de Fibonacci (F), spécifiquement les 5, 8, 13 et 21. Le nombre le plus fréquemment observé de RC dans les circonférences de tiges immatures est de 13 (53 % de toutes les tiges immatures observées), mais on en observe également 8, 21 et 5, en ordre décroissant de prévalence, respectivement. En général, plus le diamètre de la tige est grand, plus élevé est le nombre F des RC. Cependant, l'ordre des ramifications semble avoir le plus grand effet sur le nombre de RC. Spécifiquement, la pousse terminale (axe de 1°) possède généralement 21 RC dans son bourgeon terminal, et 13 dans ses bourgeons latéraux. Pour tous les autres axes terminaux (c.-à-d. 2°, 3° et 4°), la tendance est de 13, alors que chez les bourgeons latéraux associés elle est de 8. On observe également la même relation générale entre les nombres de RC, le diamètre de la tige et l'ordre des ramifications chez les ramètes matures (lignifiées) du pin lodgepole, par exemple le nombre F de RC le plus fréquemment observé est de 13 (c.-à-d. chez 48 % des ramètes observées). Les auteurs ont également évalué la chiralité phyllotactique d'échantillons de branches matures. Un arbre a tendance à retenir une chiralité (ou sens de rotation) phyllotactique donnée, à mesure que l'ordre de ramification augmente, en dépit d'une diminution du nombre de RC, allant de 21 à 5, selon les ordres de ramification allant des plus faibles aux plus élevés. Les auteurs discutent l'implication de ces constatations sur le développement phyllotactique hélicoïdal, chez les conifères.

Mots clés : nombres de Fibonacci, canaux résinifères, pin lodgepole, *Pinus contorta* var. *latifolia*, phyllotaxie.

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Introduction

Fibonacci (F) numbers, that is, those numbers belonging to the primary number series 0, 1, 1, 2, 3, 5, 8, 13, 21... or to any other series obeying the same rule of propagation and beginning with any two numbers, have been widely observed in biological forms, including plants (see Jean 1994). Fibonacci numbers are amply found in conifer species with helical phyllotaxis such as pine. Spiral phyllotaxis, the ordered spiral generation of new plant parts in the stem (typically 137.5° apart: this is known as the golden angle and is a geometrical outcome of F numbers), is particularly noticeable in many conifer species (Namboodiri and Beck 1968), including species of pine (Fredeen et al. 2002). For example, an emergent property of the F number-derived helical phyllotaxis is the two opposing diagonal files of plant parts (seen in needle fascicles or cone-scales) called parastichies that add up to sequential F numbers in a circumference, respectively.

Normal (i.e., nontraumatic or constitutive) longitudinal resin canals (RCs) are a prevalent feature among conifers, widely occurring in root, stem, and foliage (Hanes 1927), and serving as a primary defense against many associated pests (Boucher et al. 2001; O'Neill et al. 2002). In the *Pinaceae*, RCs are a relatively constant feature of the wood of *Pinus*, *Picea*, *Larix*, and *Pseudotsuga* in contrast to the other genera in the *Pinaceae* (Hanes 1927). Among these four genera, *Pinus* is considered to have RCs that are the least responsive to external factors (see Werker and Fahn 1969).

Two major lineages of pines are currently justified: the subgenus *Strobus* containing the "soft" or haploxyton pine species with a single vascular bundle in the needle, and the subgenus *Pinus* containing the "hard" or diploxyton pine species with two vascular bundles in the needle (Price et al. 1998). Of the previous studies that have examined longitudinal RCs in hard pines, that of Suzuki (1979) is of relevance to the present study. Suzuki (1979) examined eight hard and soft pines including *Pinus contorta* var. *latifolia*. His results, though based on extremely small sample sizes ranging from 1 to 4 shoots per species, included counts of 5, 8, and 13 longitudinal RCs in one species, and 13 in the juvenile pine seedlings of the *Pinus* group. However, no mention was made of F numbers, perhaps due to the limited sample size.

Suzuki (1979) also observed strong 1:1 correlations between the number of RCs and the number of vascular bundles across many of the hard pines, and Werker and Fahn (1969) provided strong evidence of this relationship for one species of old-world hard pine, *Pinus halepensis*. While vascular bundles in the primary stem are difficult to enumerate, RCs are not, making them a potentially useful indicator of vascular bundle number in many pines.

We measured the numbers of RCs in various branch orders (i.e., 1°, 2°, 3°, and 4°) of immature and mature stem of a hard pine native to western North America, lodgepole pine (*Pinus contorta* Dougl. var. *latifolia*), and related these to either stem order or diameter. Finally, based on an assumption of a 1:1 relationship between the number of RCs and vascular bundles in the primary stems of lodgepole pine, and the known relationships among number of vascular bundles, leaf trace divergence, and phyllotactic chirality (Namboodiri and

Beck 1968; Fredeen et al. 2002), we examined how phyllotactic chirality is propagated in lodgepole pine. Theory would predict that in pine, transitions between successive F numbers of vascular bundles (in the absence of changes in phyllotactic pattern or jugy; Zagórska-Marek 1985; Jean 1994) would result in one of two outcomes: (1) reversal of leaf trace divergence or (2) reversal of phyllotactic chirality (Namboodiri and Beck 1968). We sought to provide evidence that would support one of these two possibilities.

Materials and methods

Study area and sampling procedures

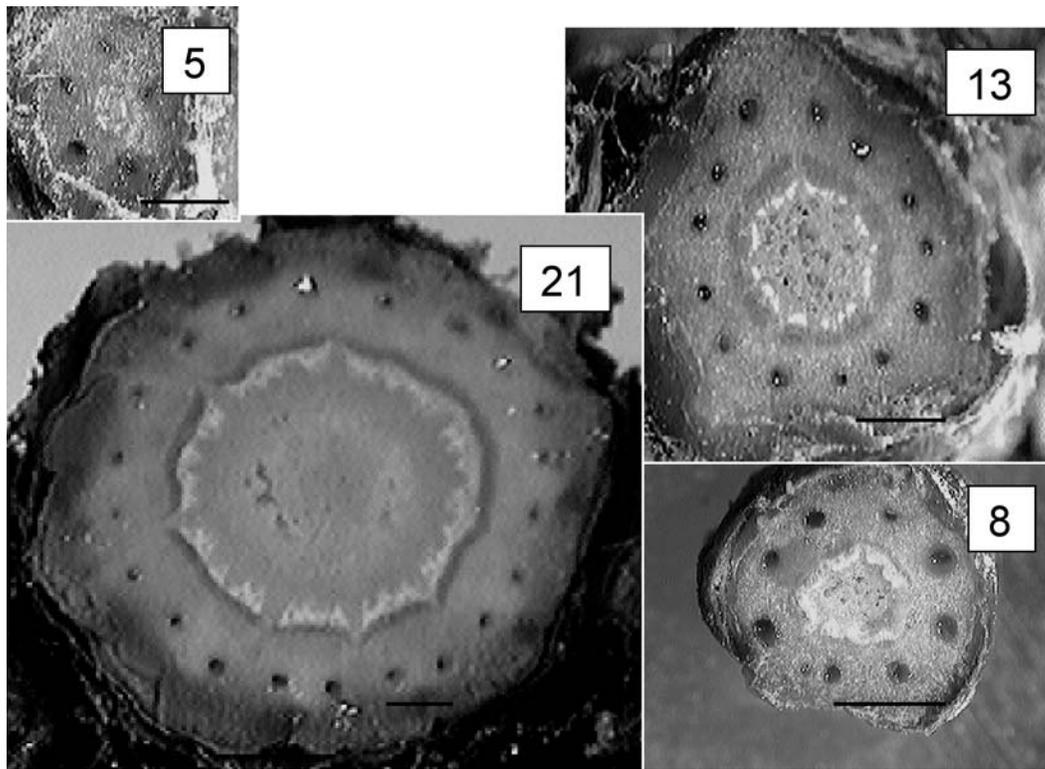
Lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) trees were located within a 2-km radius of the University of Northern British Columbia, Prince George, British Columbia (54°05'N, 122°50'W). Terminal immature stems (1°, 2°, 3°, 4°, and 5° order branches) and their associated laterals (2°, 3°, 4°, 5°, and 6° branches, respectively) were sampled in lodgepole pine trees aged 17 to 25 years with a mean age of 22. Immature stems were defined as those that were mostly green (chlorophyll-containing), poorly lignified, and rapidly expanding new stems. We define the 1° axis as the vertical leader, 2° axes as those branching off of the 1° axis, and so on. Fifth and sixth order stems were rarely found and so were excluded from the final analyses.

Immature stem samples were taken between 1 June and 10 July 2001 and refrigerated at 3 °C for a maximum of 2 weeks prior to processing. Immature stems were sectioned transversely, from the base of the bud to the apex using a sharp razor-blade and examined under a maximum magnification of 40× with an Olympus SZ40 dissecting microscope connected to an Hitachi KP-D40 color digital camera. Images were captured using Screen Machine 2.0 (Fast Multimedia AG, Munich, Germany) and evaluated for the number of primary normal longitudinal resin canals (RCs) in a circumference and the diameter of the immature stem with a stage micrometer. Care was taken not to count foliar resin canals, which emanate from the longitudinal canals. In addition, irregular or non-F numbers of resin canals were observed in a small fraction of the cases (<5% of the samples), often in zones of transition to higher F numbers. These latter samples were excluded from the comparisons made between RC number transitions and phyllotactic chirality.

Seven to 10 mature 2° and 3° order cone-bearing branchlet samples, typically 1 year of age, were sampled from each of 10 lodgepole pine trees ranging in age from 17 to 25 years in the same area as above. Both terminal and lateral cones and branches were assessed in all samples. Branch samples were evaluated for number of RCs (could not be determined for cones), average diameter of stem, and phyllotactic chirality (e.g., clockwise or counterclockwise) of the acropetal (ascending) ontogenetic spiral of needle-fascicles on branches as well as cone-scales of cones (see Fredeen et al. 2002).

The χ^2 analysis was performed as in Fredeen et al. (2002). Specifically, our assumed null hypothesis for frequency of chirality changes between different orders of branches was 50:50, that is, that the chirality of lateral branch phyllotaxis would be unrelated to the phyllotactic chirality of the subtending lower order branch.

Fig. 1. Representative images of transverse sections of immature *Pinus contorta* var. *latifolia* stem prior to or immediately after bud-break. Fibonacci number of resin canals are indicated for each image and an arrow in the bottom right of the figure indicates a resin canal. Sections shown were taken at $\times 40$ magnification under a dissecting microscope. Scale is indicated for each image by a solid black line 1 mm in length. For mean dimensional attributes of immature stems, see Fig. 3.



Results

Transverse sections of lodgepole pine immature stems typically had Fibonacci (F) numbers (i.e., 5, 8, 13, or 21) of primary normal (nontraumatic) longitudinal resin canals (RCs) in a circumference (Fig. 1). The most commonly observed number across all immature stem samples was 13 (52.8%), with 8 and 21 being the next most common numbers, respectively (Fig. 2). The number of RCs were generally found to decrease to the next lower F number from terminal to lateral immature stems, with 1° terminal immature stems (leader) segments typically having 21 RCs and all other orders of terminal branches most commonly having 13, with the occasional 2° terminal axes exhibiting 21 RCs (Fig. 2).

In general, the F number of RCs increased with branch diameter (Fig. 1). To examine this quantitatively, we plotted number of RCs against mean diameter of immature stem samples after pooling samples by either branch order (1° , 2° , 3° , and 4° , for both terminal and lateral immature stems separately; Fig. 3A) or by number of RCs (5, 8, 13, or 21; Fig. 3B). In either case, the correlation coefficients for the linear regressions between diameter and number of RCs were high with R^2 values of 0.99, 0.96, and 0.95 for upper- to lower-most relationships, respectively, (Fig. 3), though it should be noted that in one case the relationship was based on only 3 values.

Across all terminal and lateral stems ($n = 285$) of mature lodgepole pine, 48% had 13 RCs, 39% had 8, 12% had 21, and 1% had 5. In terminal branch to lateral branch and

terminal branchlet to attached cone transitions, chirality of phyllotaxis was maintained in the vast majority of the transitions (χ^2 tests, $p < 0.001$), from a low of 67% of needle to cone transitions in 2° order branches to a high of 90% in 3° order branch to branch transitions (Table 1). Among the terminal 2° and 3° axes, 73% had 13 resin canals, 18% had 21 resin canals, and the remainder had intermediate numbers.

Mature 2° order stems had a mean diameter of 7.1 ± 1.8 mm and 16.6 ± 3.7 longitudinal resin canals in a circumference, while mature 3° order stems had a smaller mean diameter of 5.9 ± 1.8 mm and only 13.6 ± 2.1 resin canals (Table 2). Lateral branches of 2° and 3° terminal branches had smaller diameters and fewer resin canals than terminals, respectively (Table 2). Over all mature terminal to lateral axis transitions ($n = 96$), the majority (75%) involved transitions from 13 RCs in the primary axis to 8 RCs in the laterals, with the only other transition of consequence (21% of the cases) involving transitions from 21 to 8 RCs.

Discussion

Primary normal longitudinal resin canals (RCs) in immature and mature stems of lodgepole pine were found to occur in F numbers of the primary series (specifically 5, 8, 13, and 21), with 13 being the most common (53% of immature stems and 48% of mature stems) and 5 being the least common. To our knowledge, this observation has not been conclusively shown for any conifer. Indications of RC numbers from the F number series (specifically 5, 8, and 13) were

Fig. 2. The Fibonacci numbers of primary longitudinal resin canals were influenced by branch order in immature stems of *Pinus contorta* var. *latifolia*. The primary (1°) axis of the seedling or tree is the leader, while subsequent axes are of increasing branching order. In each stem sample taken, the terminal (T) axis was assessed as well as the next higher order of branching that were termed laterals (L). Percentage of the first and second most frequent Fibonacci numbers of normal resin canals at each branch position are shown in parentheses.

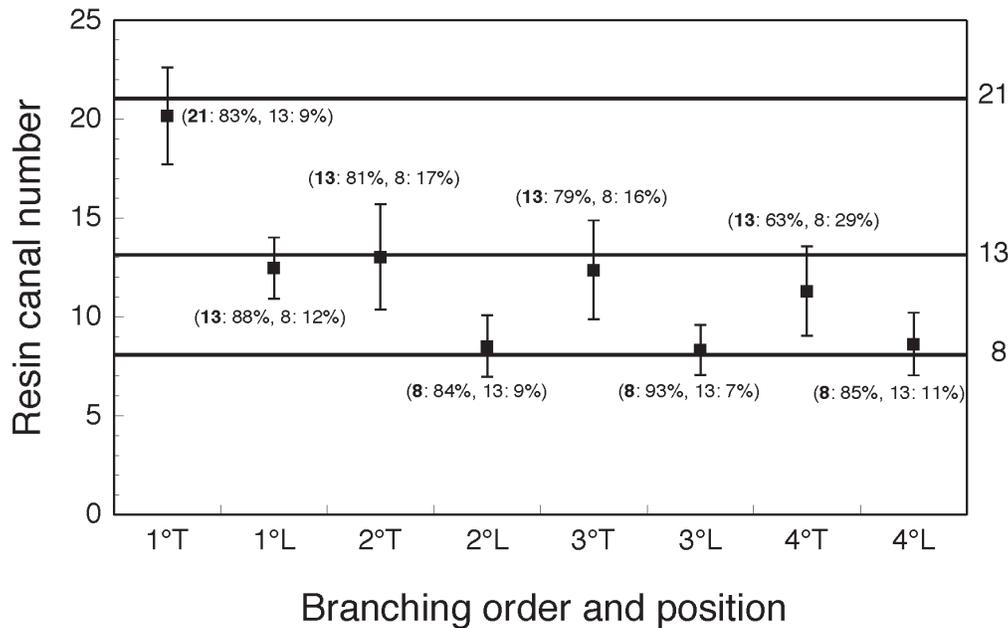


Table 1. The degree of correspondence between the chirality of phyllotaxis in 2° and 3° branch needle-fascicles (N) and that of associated lateral branchlets (3° or 4°, respectively) or cones (C) in 10 trees of *Pinus contorta* var. *latifolia* (7 to 10 samples of 2° and 3° branches per tree).

Phyllotaxis comparison	Sample size (n)	No. with same chirality	Similarity (%)	χ^2	p value (v=1)
N-N (2° to 3°)	52	40	77	34	<0.001
N-C (2°)	84	56	67	47	<0.001
N-N (3° to 4°)	61	55	90	50	<0.001
N-C (3°)	104	94	90	86	<0.001
N-N (total: 2° and 3°)	113	95	84	83	<0.001
N-C (total: 2° and 3°)	188	150	80	127	<0.001

Note: H_0 , χ^2 : chirality of lateral cone and branch phyllotaxis are not related to the chirality of the subtending meristem, that is, expected frequency of similarity is 50:50.

Table 2. Number of resin canals, stem diameter, and predicted and observed reversals between mature 2° and associated laterals (3° N), and 3° order stem samples and associated laterals (4° N) from ten 17- to 25-year-old *Pinus contorta* var. *latifolia* trees (7 to 10 samples of 2° and 3° branches per tree).

Phyllotaxis sample	Mean no. of resin canals in a stem diameter	Mean stem diameter (mm)
2° N main axis (n=77)	16.6±3.7	7.1±1.8
3° N (2° N laterals) (n=59)	8.3±1.2	3.2±1.1
3° N main axis (n=100)	13.6±2.1	5.9±1.8
4° N (3° N laterals) (n=61)	7.8±0.7	3.4±1.2

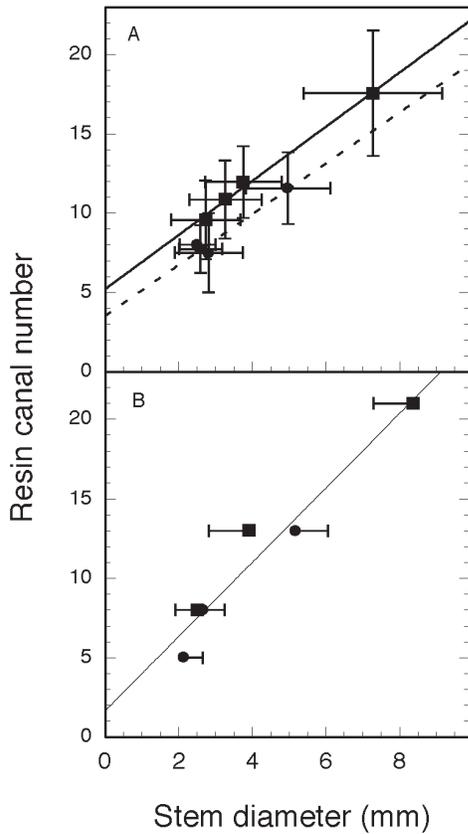
Note: Means ± SEM are shown.

indicated for various *Pinus* species by (Suzuki 1979), but perhaps the low sample sizes used in his study ($n = 1-4$) precluded generalization. More recent papers have also reported "near F number" mean numbers of RCs in stems of conifers, for example, Sitka × White spruce hybrids (e.g., 13.5 to 13.8; O'Neill et al. 2002) and white pine (11.4 ± 2.8 ;

Boucher et al. 2001), but again, no mention of F numbers and no detailed characterization of RC numbers across orders of branching were made.

Branch order had a large apparent effect on the number of RCs in immature stems of lodgepole pine. Specifically, the leader (1° axis) generally had 21 RCs in the terminal bud

Fig. 3. The correlation between mean immature stem diameter (DIAM) and mean number of primary longitudinal resin canals in a circumference (RCN) was determined after the data were grouped either by (A) branch order (1°, 2°, 3°, and 4°) or by (B) Fibonacci number of resin canals (i.e., 5, 8, 13, or 21). ■, terminal (T) immature stem means ± SD along both axes; ●, lateral (L) immature stem means ± SD along both axes. In the case of branch order, two separate linear regression equations are shown, one for terminal immature stem resin canal number ($RCN_{term} = 1.71 * DIAM_{term} + 5.21; R^2 = 0.99$) and another for lateral immature stem resin canal number ($RCN_{lat} = 1.60 * DIAM_{lat} + 3.53; R^2 = 0.96$). In the case where data were grouped by number of resin canals (Fig. 3B), a single linear regression for all data is shown ($RCN = 2.34 * DIAM + 1.65; R^2 = 0.95$).



with 13 in lateral (2°) buds (Fig. 2). All other terminal axes (i.e., 2°, 3°, and 4°) tended to 13, with associated lateral axes tending to 8. The same general relationships between numbers of RCs and branch order were also observed for mature (woody) branchlets of lodgepole pine (e.g., Table 2). Of the previous studies that have enumerated RCs in pine, none have examined the variation in numbers between branch orders. Early work of Hanes (1927) was suggestive of increasing RC numbers with stem age, but no quantitative analysis or specific relationships were provided.

Mean numbers of RCs in both terminal and lateral stems were linearly and positively correlated with cross-sectional immature stem diameter (R^2 values = 0.95), either after grouping by branch order (Fig. 3A) or by F number of resin canals (Fig. 3B). Thus, while branching order is clearly a strong determinant of RC number, we propose that branch diameter may have a lesser secondary role. Since diameter

naturally decreases as branching order increases, we must base this conclusion largely on the detailed developmental information contained in papers by Hanes (1927), Werker and Fahn (1969), and Suzuki (1979).

Phyllotactic chirality was also assessed for the mature branch needle and cone samples. Lodgepole pine branches tend to conserve a phyllotactic chirality (or handedness) through branch order transitions (see Fredeen et al. 2002; Table 1), despite transitions in RC number from a high of 21 to a low of 5 from low to high branch order, respectively (Tables 1 and 2). In fact, the majority of 2° and 3° branch to lateral transitions exhibited reductions in RCs from 13 in the main axis to 8 in laterals, with the remainder largely being 21 to 8 transitions (e.g., Fig. 2). The consequences of this finding are of potential importance to the documented relationship between phyllotactic chirality, leaf trace divergence, and number of vascular bundles first formalized by Namboodiri and Beck (1968), with the assumption that RC numbers are indeed equivalent to vascular bundle number in lodgepole pine.

We were not able to establish a definite correspondence between number of RCs and number of vascular bundles in lodgepole pine stems in this study. Vascular bundles are difficult to enumerate because they are both in close proximity with frequent contact zones and have diffuse boundaries, and because diverging needle-traces can typically be confounded with the vascular bundles. However, we will assume a 1:1 relationship between number of RCs and vascular bundles in lodgepole pine based on the following evidence. First, Suzuki (1979) observed strong 1:1 correlations in number of primary longitudinal resin canals with number of vascular bundles across various hard pines, and Werker and Fahn (1969) provided detailed evidence of this relationship for a species of old-world hard pine, *Pinus halepensis*. Second, Namboodiri and Beck (1968) observed 13 (followed by 8) to be the most commonly observed numbers of vascular bundles in members of the *Pinaceae*, similar to the relative prevalences of 13 and 8 RCs in lodgepole pine immature and mature stems demonstrated in this study. Finally, we observed a qualitative drop in the number of vascular bundles or leaf traces that was concurrent with the drop in numbers of RCs with branch diameter (e.g., Fig. 1).

The chirality of the phyllotactic ontogenetic spiral (the direction in which new needle and cone-scales are produced in meristems—and evident later in stems) can be predicted from (1) the number of vascular bundles, (2) the angle of divergence, and (3) the direction (or chirality) of needle-trace divergence from the vascular bundles (see Namboodiri and Beck 1968). The relationship is further simplified because the angle of divergence is 137.5° in lodgepole pine (see Fredeen et al. 2002). Therefore, the simplified relationship can be restated as the following: when vascular bundles number 5 or 13, the chiralities of phyllotactic spiral and that of leaf trace divergence are the same (concordant) and when the vascular bundles number 8 or 21, the chiralities are opposite (discordant; Fredeen et al. 2002). Thus, if number of vascular bundles (i.e., number of RCs) changes from F_n to F_{n-1} or F_{n+1} , two scenarios are possible: (i) the chirality of the phyllotactic spiral remains unchanged while chirality of leaf trace divergence reverses or (ii) chirality of leaf trace divergence remains unchanged while the chirality of phyllo-

taxis reverses. While we have not measured leaf trace divergence in this paper, we have measured phyllotaxis, and therefore can make inferences about leaf trace divergence. Our results support the first mechanism. Specifically, we generally observed a decrease in number of RCs by one F number from terminal to lateral branchlets in 2° and 3° immature stems (Fig. 2) and in mature stems of lodgepole pine (e.g., Table 2), while chirality of phyllotaxis was significantly and highly conserved (from 67 to 90% of cases) across these same stem order transitions (Table 1). A strong tendency towards propagation of a given chirality within a lodgepole pine tree (Fredeen et al. 2002), along branch axes (A. Fredeen unpublished, and in *Abies balsamea* (Zagórska-Marek 1985)), or between branch orders (Fredeen et al. 2002) have been observed previously and are supportive of the first mechanism. Direct measurement of leaf trace divergence chirality reversals across such transitions in RC number will be needed to positively confirm this hypothesis.

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