

Flower and seed production as affected by axis category and shoot size in two Patagonian *Nothofagus* species

Cristian D. Torres, Javier G. Puntieri, and Marina Stecconi

Abstract: Flower distribution within the tree crown may affect both pollination dynamics and the costs of flowering on tree growth. For *Nothofagus obliqua* (Mirb.) Oerst. and *Nothofagus nervosa* (Phil.) Krasser, the production of flowers and viable seeds were compared among axes of contrasting size and category in the tree crown. For annual shoots arising from three axis categories (main branches, secondary branches, and twigs), the numbers of nodes, staminate flowers, pistillate flowers and axillary buds, and seed viability were evaluated. Most flowering shoots produced both staminate and pistillate flowers. The number of flowers of each type was related positively with the number of nodes of the shoot. Longer shoots had proportionally more pistillate flowers than short shoots. The three axis categories produced both flower types in direct proportion to the size of their shoots, but main branches presented more axillary vegetative buds than secondary branches and twigs. For *N. obliqua*, the percentage of viable seeds was lower in secondary branches than in main branches and twigs, perhaps owing to a lower probability of cross-pollination in secondary branches. The coexistence of vegetative and reproductive functions may cause interference between them. Massive allocation of meristems to flowering reduces meristem availability for growth and, especially, flowering in the following growing season.

Key words: annual shoot, axis category, staminate flower, pistillate flower, axillary bud, seed viability.

Résumé : La distribution des fleurs au sein de la cime des arbres peut affecter la dynamique de la pollinisation et le coût de la floraison pour l'arbre. Les auteurs ont comparé chez le *Nothofagus obliqua* (Mirb.) Oerst. and *Nothofagus nervosa* (Phil.) Krasser, la production des fleurs et des graines viables chez des rameaux de dimensions et de catégories contrastées dans le houppier. Chez les tiges annuelles provenant de trois catégories d'axes (rameaux principaux, rameaux secondaires, et ramilles) les auteurs ont évalué les nombres de nœuds, de fleurs staminées, de fleurs pistillées et de bourgeons axillaires, ainsi que la viabilité des graines. On observe une relation positive entre le nombre de fleurs de chaque type avec le nombre de nœuds sur une tige. Les rameaux les plus longs montrent une plus forte proportion de fleurs pistillées que les rameaux courts. Les trois catégories d'axes produisent les types de fleurs en proportion directe avec leurs dimensions, mais les rameaux principaux présentent plus de bourgeons végétatifs axillaires que les rameaux secondaires et les ramilles. Chez le *N. obliqua*, on observe un plus faible pourcentage de graines viables chez les rameaux secondaires que chez les rameaux principaux et les ramilles, peut-être dû à une probabilité plus faible de pollinisation croisée chez les rameaux secondaires. La coexistence des fonctions reproductives et végétatives peut causer une interférence entre elles. Une allocation massive aux méristèmes pour la floraison réduit la disponibilité des méristèmes pour la croissance, surtout la floraison au cours de la saison de croissance suivante.

Mots-clés : tiges annuelles, catégorie d'axes, fleur staminée, fleur pistillée, bourgeon axillaire, viabilité des graines.

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Introduction

In most tree species the crown consists of a hierarchical branching system with different axis categories that may be defined by topological, morphological, anatomical, physiological, and functional features (Tomlinson 1978; Barthélémy et al. 1999; Puntieri et al. 2003; Puntieri and Ghirardi 2010). In architectural studies, tree axes are categorized mainly

based on topology, size, and morphological features such as phyllotaxis, number of leaves, and stem orientation and branching. The level of differentiation of tree axes is considered to increase from the most vigorously growing axes, such as the trunk and the main branches, to the least vigorous axes, such as brachyblasts and reproductive axes (Barthélémy and Caraglio 2007). In many species, reproductive structures are developed by a particular axis category, which is then

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considered to be the most highly differentiated axis category for the species concerned (Grosfeld et al. 1997; Suzuki 2000; Ishihara and Kikuzawa 2004; Normand et al. 2009). This may be an oversimplification in the case of tree species with reproductive structures on axes of diverse topological locations (e.g., in *Nothofagus*; Puntieri et al. 2009).

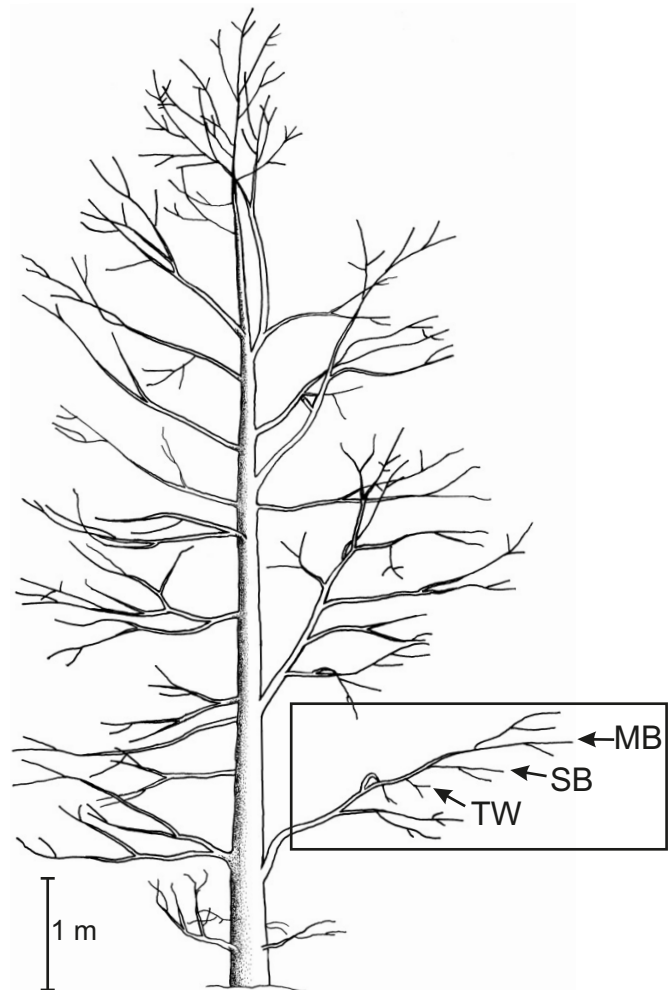
Plant growth and flower production demand resources and meristems, so that these two developmental activities may affect each other both at the individual level and at the axis level (Suzuki 2001; Kawamura and Takeda 2006; Normand et al. 2009). The distribution of flowers within each plant would be a consequence of the physiological and meristematic costs implicated in flower production, as well as selection pressures favoring cross-pollination and against selfing (Lovett Doust and Lovett Doust 1988; Dai and Galloway 2011). Reproductive success depends largely on the spatial and temporal distributions of stamens and pistils, which determine pollen flow and, therefore, the probabilities of cross- and self-pollination (Lloyd and Webb 1986; Webb and Lloyd 1986). In anemophilous tree species with unisexual flowers, reproductive success is conditioned by the relationship between the distributions of staminate and pistillate flowers in the crown and pollen dispersal.

The genus *Nothofagus* (Nothofagaceae) includes diclinomonocious, anemophilous, and self-incompatible tree species (Riveros et al. 1995a; Donoso et al. 2006a, 2006b). For young-adult trees of *Nothofagus* species from the temperate forests of South America, three axis categories arising from the trunk may be identified (Barthélémy et al. 1999; Puntieri et al. 2003; Stecconi 2006; Fig. 1). The least differentiated of such axes are the main branches, which arise directly from the trunk and cause lateral crown expansion (Fig. 1). The most differentiated axes are the twigs, which derive from any axis category in nonperipheral crown positions. Secondary branches are intermediate in differentiation and topology between main branches and twigs. These three axis categories usually differ from each other regarding the size of their shoots (with a shoot being defined as an axis portion extended during one growing season): main-branch shoots > secondary-branch shoots > twig shoots. The ages of trees and axes, as well as environmental factors, nonetheless, may add variation to shoot traits within each axis category (Puntieri and Ghirardi 2010). As a consequence, topological information allows a more precise identification of these axis categories.

In *Nothofagus*, staminate and pistillate inflorescences arise in lateral positions during the extension of flowering shoots, which also have green leaves and axillary buds. Usually, flowering shoots of *Nothofagus* have both flower types, although the number of inflorescences varies within and between individual trees (Puntieri et al. 2009). So far, we ignore the extent to which these variations are linked with the size and topology of flowering shoots. Moreover, it is also unknown if the size and topology of a flowering shoot affect its probability of viable seed production.

It has been shown for tree species that the degree of differentiation of an axis is positively related to its capacity to produce flowers (Barthélémy and Caraglio 2007). According to the gradient of axis differentiation described for *Nothofagus*, it may be hypothesized that the number of reproductive structures per annual shoot should increase following the gradient:

Fig. 1. Diagrammatic representation of a *Nothofagus obliqua* tree (about 25 years old) showing the three axis categories considered in this study: main branch (MB), secondary branch (SB), and twig (TW).



main branches < secondary branches < twigs. In contrast, considering that exogenous pollen would more likely reach the periphery of a tree than its crown centre, main-branch shoots, which integrate the crown's periphery (Stecconi et al. 2010), would be expected to produce more pistillate flowers and viable seeds than shoots of the other two, nonperipheral, axis categories. In this work we tested these ideas on two *Nothofagus* species from Patagonia.

Materials and methods

Study species and sites

Two closely related South American species, *Nothofagus obliqua* (Mirb.) Oerst. and *Nothofagus nervosa* (Phil.) Krasser (syn. *Nothofagus alpina*), were included in the present study. These are the most valued *Nothofagus* species in forestry due to their fast growth and high wood quality (Stewart 1979; Tuley 1980; Destremau 1988). In these *Nothofagus* species, staminate and pistillate flowers are arranged in trimerous unisexual inflorescences arising from flowering shoots. Most flowering shoots develop both flower types. Because of the constant number of flowers per inflorescence,

from this point onwards we will use, for brevity, the term flower instead of inflorescence. Staminate flowers develop at the basal nodes of the flowering shoots, whereas pistillate flowers derive from nodes located distally with respect to those with staminate flowers. Along a flowering shoot, nodes with staminate and pistillate flowers are mostly consecutive in *N. obliqua* and separated by one to several nodes devoid of flowers in *N. nervosa* (Puntieri et al. 2009). Nonflowering or vegetative nodes of flowering shoots of these species bear, in most cases, axillary buds with the potentiality to develop sibling shoots.

Twenty-five reproductive *N. obliqua* trees and 15 reproductive *N. nervosa* trees growing in Argentina were selected. Eighteen of the selected *N. obliqua* trees inhabit a native *Nothofagus* forest near Lacar lake (Lanín National Park; 40°09'S, 71°20'-71°33'W; 670–840 m a.s.l.) and the other individuals are planted in parks at San Carlos de Bariloche city (41°08'S, 71°10'W; 878 m a.s.l.). In the case of *N. nervosa*, eight trees are located in a native forest near Lolog lake (Lanín National Park; 40°00'S, 71°22'W; 930 m a.s.l.), and seven are planted in parks (at least 400 m from each other): six at San Carlos de Bariloche city and one at El Bolsón town (41°58'S, 71°32'W; 305 m a.s.l.). Adult trees of these species are uncommon in these two localities. All selected trees had low main branches accessible for the present study (2–3 m high from the ground). Tree ages were not determined with precision but, based on bibliographic information, ranged between 25 and 120 years (Puntieri et al. 2009). Adult trees of these species with their flowering branches accessible for sampling are difficult to find. Consequently, we decided not to set tree-age and site limits in our selection of trees.

Assessment of flower distribution

For each individual tree, we selected, at the end of the 2008–2009 growing season, one main branch with flowering shoots developed on the main branch itself, as well as on its secondary branches and twigs (Fig. 1). These three axis categories were defined based on their topological position on the crown: main branches constituted the crown's periphery, secondary branches derived laterally from the main branch and were at an intermediate position in the crown, and twigs also derived from the main branch and were closer to the trunk than secondary branches. The limits between shoots extended in different years were identified through morphological markers (Barthélémy et al. 1999). For each of the three axis categories of each tree, we sampled one shoot extended during the 2007–2008 growing season, hereinafter referred to as parent shoot. At the time of sampling, each parent shoot was bearing a set of shoots extended in the 2008–2009 growing season, referred to as sibling shoots (Fig. 2). Shoot harvesting was performed during February 2009 before natural fruit release. All shoots sampled were taken to the laboratory where the number of sibling shoots of each parent shoot and the number of nodes and axillary productions of all sibling shoots were recorded. Possible axillary productions were: staminate flower, pistillate flower, and axillary bud. The axillary production could not be determined with precision for about 1% of the nodes for different reasons (absence of meristematic cells, flower abortion, or damage to axillary organs). These nodes were excluded from the analyses. Since staminate flowers fall soon after pollen release, their presence

was determined by identifying the scars left by these flowers on the stem after their abscission. Only for those trees that were growing in natural forests, in which cross-pollination may have occurred, the fruits of each sibling shoot sampled were cut transversely to determine their viability by checking for the presence of an embryo. This analysis was not performed in urban trees because of the alleged self-incompatible nature of these species and the low probability of cross-pollination in this habitat.

Data analyses

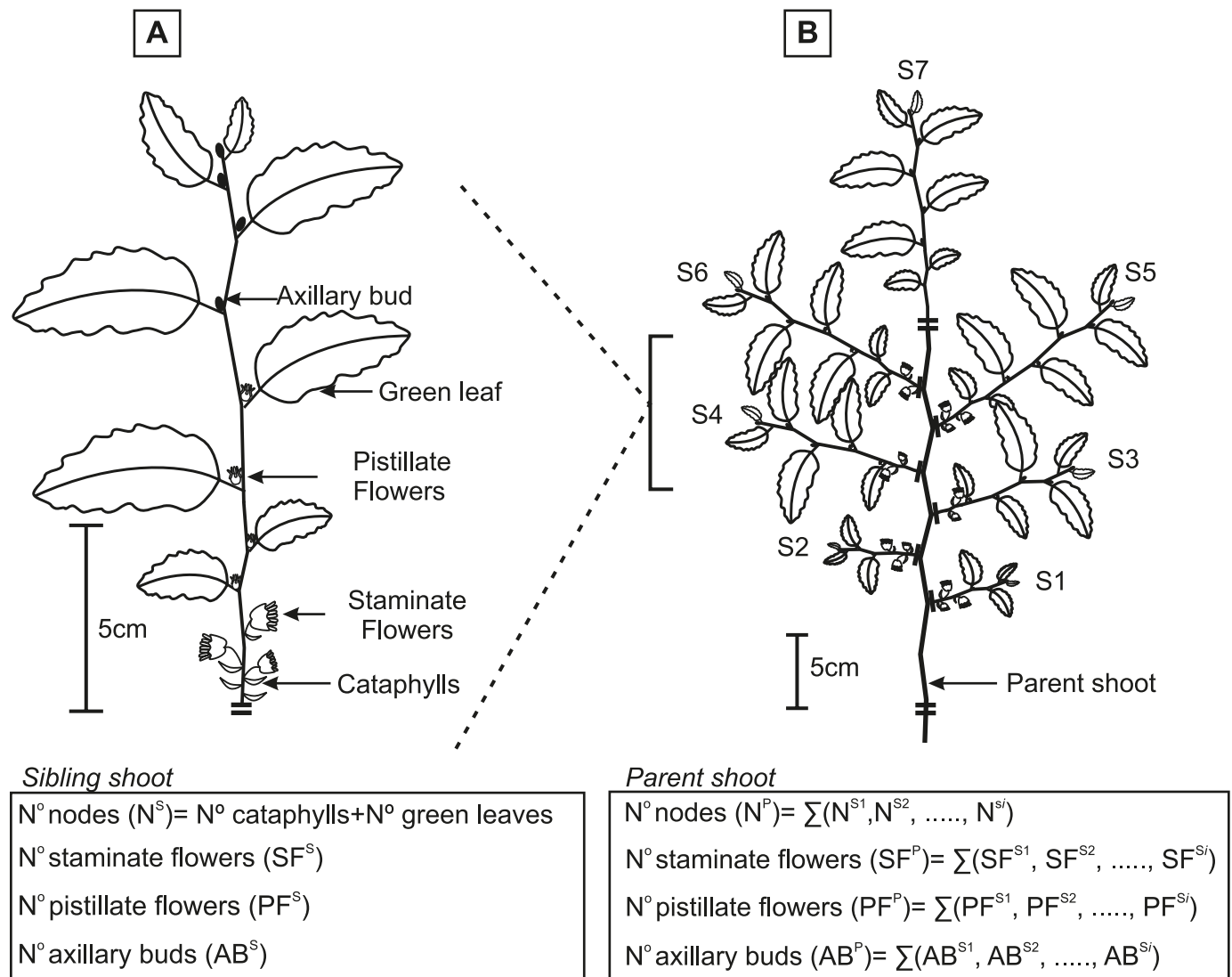
To test for morphological differences among the three axis categories defined by their topology, parent shoots of these categories were compared regarding: (i) the number of sibling shoots arising from them, and (ii) the mean number of nodes per sibling shoot. Comparisons were performed by means of one-way ANOVA. When significant differences among axis categories were observed, a posteriori Tukey's tests were applied for pairwise comparisons (Sokal and Rohlf 1981). The effect of the site of tree growth was not evaluated since neither urban areas nor natural forests were considered environmentally homogeneous. Moreover, tree ages within each site were uneven, and the geographical origin of the majority of urban trees was unknown, so that we considered it unwise to combine trees based on the sampling sites.

In *Nothofagus* spp., the sibling shoots developed from a parent shoot often exhibit a decreasing size gradient from the distal to the proximal end of the parent shoot (Puntieri et al. 2003). To account for this structural, and probably functional, interdependency among sibling shoots derived from a common parent shoot, the productions of staminate flowers, pistillate flowers, and axillary buds were evaluated on two bases: (1) sibling shoot and (2) parent shoot. The unit of analysis was, in the former case, the sibling shoot and, in the latter case, the parent shoot, which included pooled information of all corresponding sibling shoots (Fig. 2).

(1) Sibling shoots

Sibling shoots of each species were grouped in size classes based on the frequency distributions of the numbers of nodes of all sibling shoots of each species. For *N. obliqua*, sibling shoots with 8–10 nodes, 11–12 nodes, 13–14 nodes, and ≥ 15 nodes were grouped. In the case of *N. nervosa*, sibling shoots with 8–13 nodes, 14–16 nodes, and ≥ 17 nodes were grouped. The minimum number of nodes was 8 for both species. The percentages of sibling shoots that developed both flower types (henceforth bisexual shoots, BS), only staminate flowers (staminate shoots, SS), only pistillate flowers (pistillate shoots, PS), and no flowers (vegetative shoots, VS) were determined for each axis category and shoot-size class of each species. In the case of *N. nervosa*, the proportions of BS, SS, PS, and VS were compared among the three axis categories and among shoot-size classes by means of χ^2 tests (Sokal and Rohlf 1981). In the case of *N. obliqua*, since PS and VS were not present in all axis categories and shoot-size classes, χ^2 tests were applied without including these shoot types. In the following analyses those sibling shoots that did not developed flowers were excluded. Least-squares regressions on log-transformed data were performed linking the number of nodes of the sibling shoot (N^S ; predictor variable) with the numbers of staminate flowers (SF^S) or pistillate

Fig. 2. (A) Sibling shoot with staminate flowers, pistillate flowers, and axillary buds, and (B) parent shoot and its sibling shoots (S1–S7). Parallel lines indicate the proximal and distal ends of the parent shoot and shoot thick lines indicate the proximal limit of each sibling shoot. The bottom panels indicate the data registered for sibling shoots and parent shoots.



flowers (PF^S) per sibling shoot (dependent variables). In both species the frequency distribution of axillary buds (AB^S) deviated significantly from normality even after log-transformation so that the relationship between AB^S and N^S was assessed by means of Spearman's rank-correlations (ρ). SF^S and PF^S were compared among axis categories by means of analysis of covariance (ANCOVA), including axis category as fixed factor, individual tree as random factor, the interaction between axis category and individual tree, and N^S as covariable (Zar 1984). Whenever the interaction term contributed significantly to the variance (Fisher's F test; $p < 0.05$), no further analysis was performed. Otherwise, a similar ANCOVA was performed without including the interaction term. The interaction between the fixed factor and the covariable had been previously tested and excluded from the analyses owing to its lack of significance. Variables were log-transformed whenever necessary to comply with the normality and homoscedasticity required for ANCOVA. Because of

the non-normality of AB^S , comparisons among axis categories regarding this variable were made with nonparametric Kruskal–Wallis tests. The latter comparisons were performed on the relative number of axillary buds (i.e., AB^S/N^S), so as to assess the effect of axis category without considering the effect of sibling-shoot size.

(2) Parent shoots

For each parent shoot, the numbers of nodes (N^P), staminate flowers (SF^P), pistillate flowers (PF^P), and axillary buds (AB^P), including all sibling shoots, were obtained (Fig. 2B). Least-squares regressions linking N^P (predictor variable) with SF^P and PF^P (dependent variables) were computed on log-transformed data. As the data concerning AB^P showed a non-normal distribution even after log-transformation, the relationships between AB^P and N^P were assessed by means of Spearman's rank-correlations (ρ). Axis categories were compared regarding SF^P and PF^P with ANCOVA, including axis

Table 1. Mean number of sibling shoots and nodes per sibling shoot for parent shoots of *Nothofagus obliqua* and *Nothofagus nervosa*.

	MB	SB	TW	<i>F</i>	<i>p</i>
<i>N. obliqua</i> (n = 75)					
No. of sibling shoots	14.5a (1.20)	7.4b (0.81)	4.1c (0.41)	36.5	<0.001
No. of nodes per sibling shoot	14.7a (0.46)	13.0b (0.27)	11.7c (0.28)	18.4	<0.001
<i>N. nervosa</i> (n = 44)					
No. of sibling shoots	8.1a (0.87)	5.5b (0.73)	2.5c (0.27)	16.5	<0.001
No. of nodes per sibling shoot	16.6a (0.72)	15.3a (0.73)	12.7b (0.67)	7.9	0.001

Note: Mean and standard error (between brackets) are indicated for main branches (MB), secondary branches (SB), and twigs (TW). Results of one-way ANOVA (Fisher's *F* and error probability *p*) are provided. Means on the same line with different letters on the right are significantly different (Tukey's pairwise comparisons). The number of shoots (*n*) is indicated for each species.

category as fixed factor, individual tree as random factor, and N^P as covariate. In this analysis, the interaction between axis category and individual tree was not included in the model, as only one parent shoot per category and per tree was sampled (see two-factor analysis of variance without replication, Zar 1984). The interaction between the fixed factor and the covariable had a nonsignificant effect so that this interaction was excluded from the analyses. SF^P , PF^P , and the covariable were log-transformed to normalize data distributions. AB^P was compared on a relative basis (AB^P/N^P) between axis categories by means of Kruskal–Wallis tests.

Only for those trees selected in natural forests, the percentage of viable seeds produced was compared between axis categories with a χ^2 test.

All statistical analyses were performed with the R statistical program (R Development Core Team 2008); a 5% significance level was adopted.

Results

Differences among axis categories

In both species, parent shoots of the three axis categories differed regarding the number of sibling shoots arising from them and the mean number of nodes per sibling shoot (Table 1). Parent shoots of main branches had more sibling shoots than those of secondary branches that, in turn, had more sibling shoots than those of twigs. The mean number of nodes per sibling shoot was also higher for main branches than for the other two axis categories in *N. obliqua*. In the case of *N. nervosa*, the number of nodes per sibling shoot was similar for main branches and secondary branches and higher for any of these than for twigs (Table 1).

Distributions of flowers and flowering shoots

Sibling shoots

In both species, BS were more frequent than SS, PS, and VS, and SS were more abundant than VS and PS (Fig. 3). The proportions of BS, SS, PS, and VS in *N. nervosa*, and the proportions of BS and SS in *N. obliqua*, were independent of axis category ($\chi^2 = 5.9$, *df* = 6, *p* = 0.430, and $\chi^2 = 2.3$, *df* = 2, *p* = 0.316, respectively; Figs. 3A, 3B). In the case of *N. obliqua*, VS were observed only on main branches, and the proportions of BS and SS were not related to shoot size ($\chi^2 = 5.2$, *df* = 3, *p* = 0.160; Fig. 3C). For *N. nervosa*, the proportion of BS was higher and the proportions of SS and VS lower among long shoots than among short and intermediate-size shoots ($\chi^2 = 22.6$, *df* = 6, *p* <

0.001); the proportion of PS did not vary with shoot size (Fig. 3D).

A positive relationship with N^S was found for SF^S in the case of *N. obliqua*, and for PF^S in both species (Table 2; Figs. 4A, 4B). The log-log regression coefficients (*b*) indicated a less than proportional increase in SF^S with N^S in *N. obliqua*, and more than proportional increases in PF^S with N^S for both species (Table 2). A slight tendency toward more AB^S in long than in short shoots was evidenced by the positive correlations between AB^S and N^S found for *N. obliqua* ($\rho = 0.65$, *p* < 0.001) and *N. nervosa* ($\rho = 0.22$, *p* < 0.001; Figs. 5E, 5F). After accounting for the effect of the number of nodes and the individual tree neither SF^S nor PF^S differed significantly among axis categories for either species (Table 3; Figs. 5A–5D). The individual tree contributed significantly to the variations in SF^S and PF^S for both species, whereas the interaction between axis category and individual tree also affected the variations in SF^S and PF^S for *N. obliqua* (Table 3). The relative number of AB^S decreased from main-branch to twig shoots in both species (Kruskal–Wallis test: *p* < 0.001).

Parent shoots

SF^P and PF^P were positively related with N^P in both species (Table 2; Figs. 4C, 4D). The slopes of the log-log regressions indicated that SF^P increased less than proportionally with the increase in N^P for both species; PF^P increased proportionally with N^P in *N. obliqua*, and less than proportionally in *N. nervosa* (Table 2). The correlation between AB^P and N^P was positive and significant both in *N. obliqua* ($\rho = 0.92$, *p* < 0.001) and *N. nervosa* ($\rho = 0.91$, *p* < 0.001). After accounting for the effects of N^P and the individual tree, no differences among axis categories regarding SF^P and PF^P were detected for either species (Table 4; Fig. 6). The contribution of individual trees to the variation in the number of flowers was significant only in the case of PF^P of *N. nervosa* (Table 4). The relative number of AB^P was higher for main branches than for secondary branches and twigs for both species (Kruskal–Wallis test: *N. obliqua*, *p* < 0.001; *N. nervosa*, *p* = 0.004; Fig. 6). Secondary branches and twigs presented similar proportions of AB^P in the case of *N. obliqua*, whereas in the case of *N. nervosa* secondary branches had a higher proportion of AB^P than twigs (Fig. 6).

Production of viable seeds

For *N. obliqua*, a lower proportion of viable seeds was produced in secondary-branch shoots than in shoots of the

Fig. 3. Percentages of shoots that developed only staminate flowers (staminate shoots, SS), only pistillate flowers (pistillate shoots, PS), both flower types (bisexual shoots, BS), and no flowers (vegetative shoots, VS) for different axis categories (A, B) and different shoot sizes (C, D) for *Nothofagus obliqua* (A, C) and *N. nervosa* (B, D). At the top of the bars is indicated the number of shoots of each axis category and shoot size. Codes of axis categories like in Fig. 1.

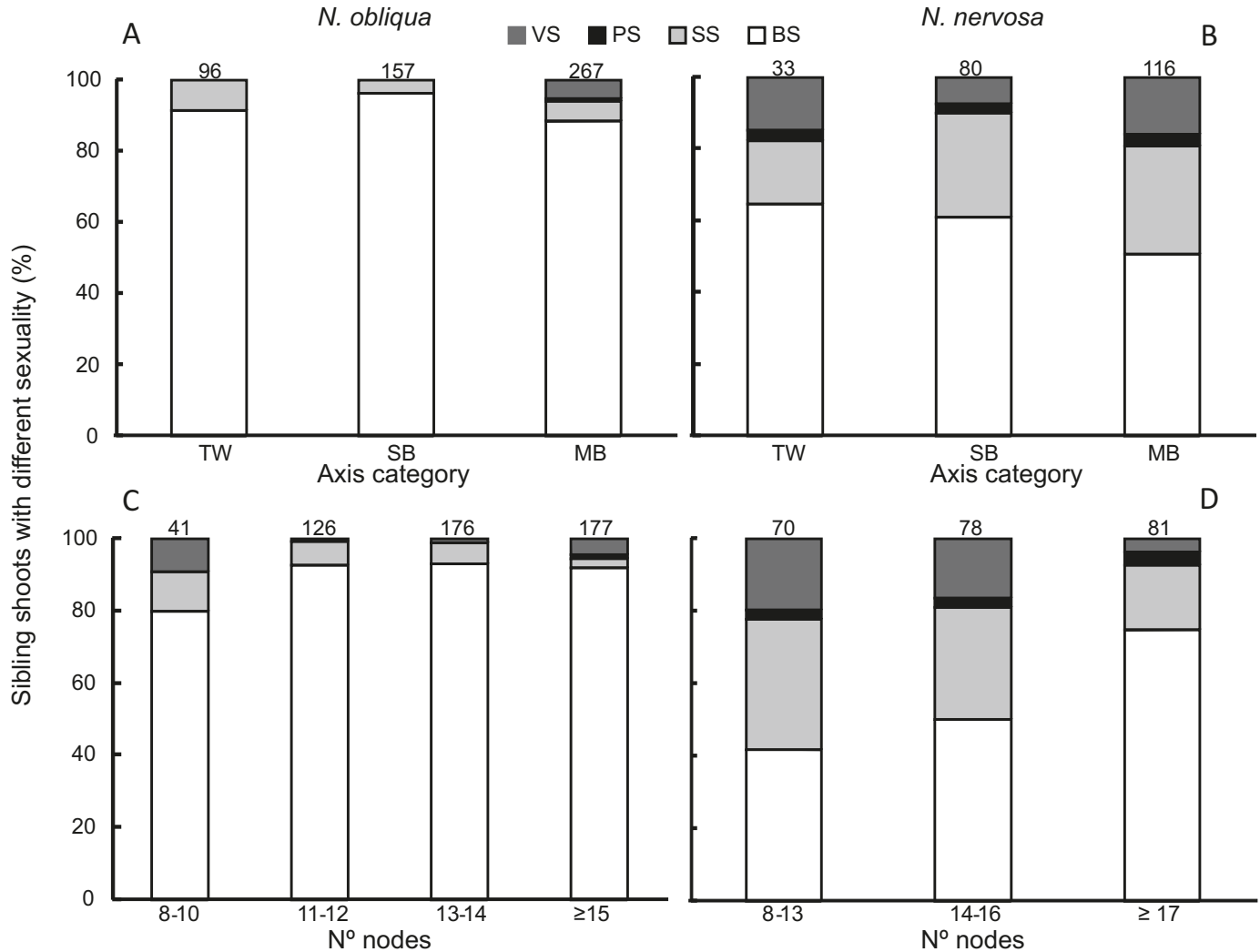
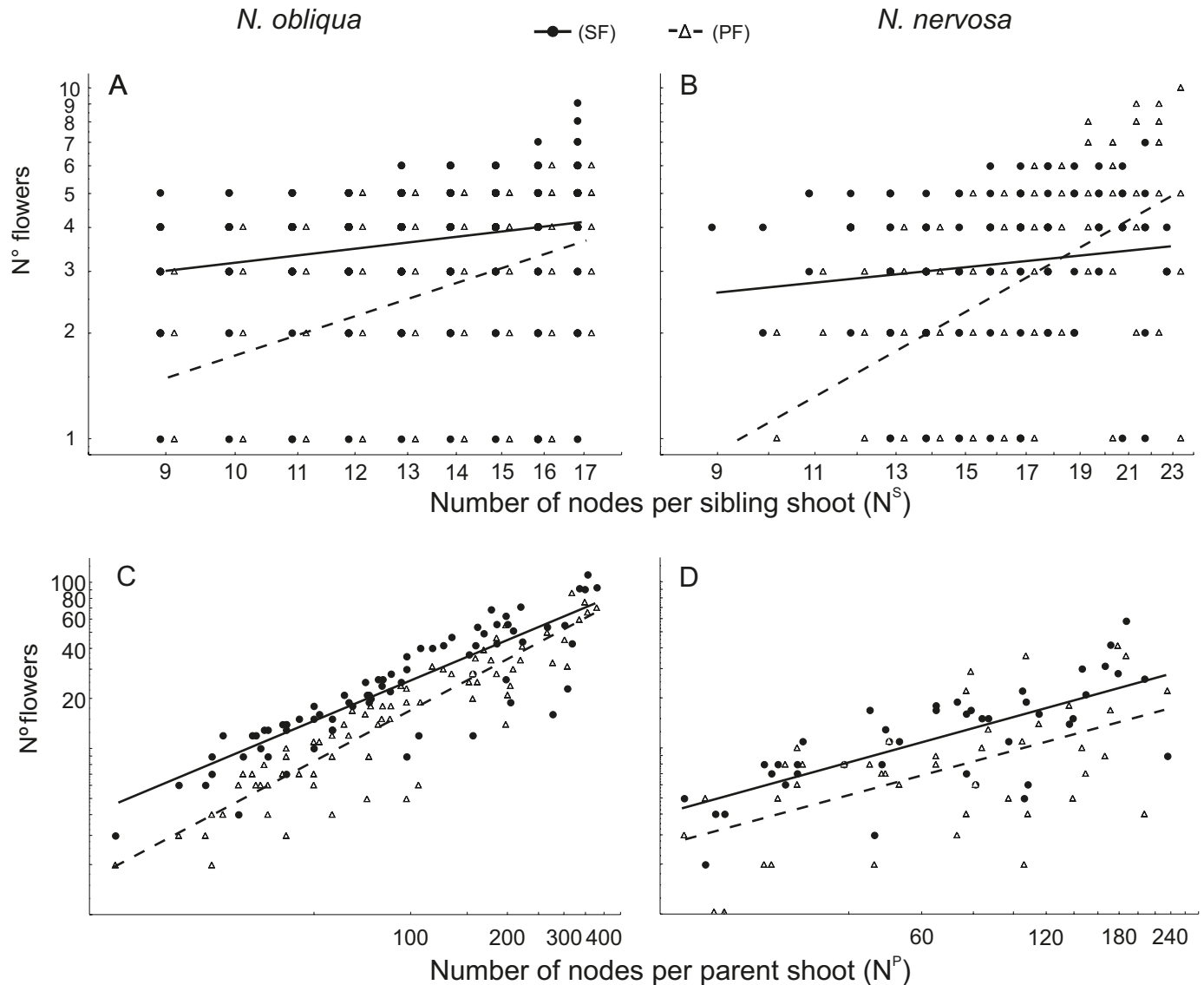


Table 2. Relationships between number of nodes and numbers of flowers per sibling shoot and parent shoot (all variables log-transformed).

Species	Regression	<i>b</i>	-95% CL	+95% CL	<i>p</i>	<i>R</i> ² (%)
Sibling shoots						
<i>N. obliqua</i> (<i>n</i> = 490)	N ^S -SF ^S	0.50	0.31	0.79	<0.001	5.2
	N ^S -PF ^S	1.42	1.21	1.63	<0.001	26.9
<i>N. nervosa</i> (<i>n</i> = 133)	N ^S -SF ^S	Not significant				
	N ^S -PF ^S	1.79	1.35	2.23	<0.001	32.7
Parent shoots						
<i>N. obliqua</i> (<i>n</i> = 75)	N ^P -SF ^P	0.83	0.73	0.93	<0.001	76.4
	N ^P -PF ^P	1.01	0.90	1.12	<0.001	83.7
<i>N. nervosa</i> (<i>n</i> = 44)	N ^P -SF ^P	0.69	0.48	0.90	<0.001	51.7
	N ^P -PF ^P	0.68	0.37	0.99	<0.001	32.1

Note: Linear regression coefficients (*b*) and their upper and lower 95% confidence limits (CL) for the log-log relationships between the number of nodes (*N*; independent variable) per shoot and the numbers of staminate (SF) and pistillate flowers (PF; dependent variables) for sibling shoots (—^S) and parent shoots (—^P) of *Nothofagus obliqua* and *N. nervosa*. Number of shoots (*n*), error probability (*p*), and coefficient of determination (*R*²) are indicated for each regression.

Fig. 4. Relationships between the number of nodes (N) and the numbers of staminate (SF; solid circles, continuous lines) and pistillate flowers (PF; open triangles, dashed lines), for sibling shoots (A, B) and parent shoots (C, D) of *Nothofagus obliqua* and *N. nervosa*. Both axes are presented in logarithmic scale. See regression details in Table 2.



other two axis categories ($\chi^2 = 20.9$, $df = 2$, $p < 0.001$; Fig. 7). In the case of *N. nervosa*, all axis categories included similar proportions of viable seeds ($\chi^2 = 0.7$, $df = 2$, $p = 0.707$; Fig. 7).

Discussion

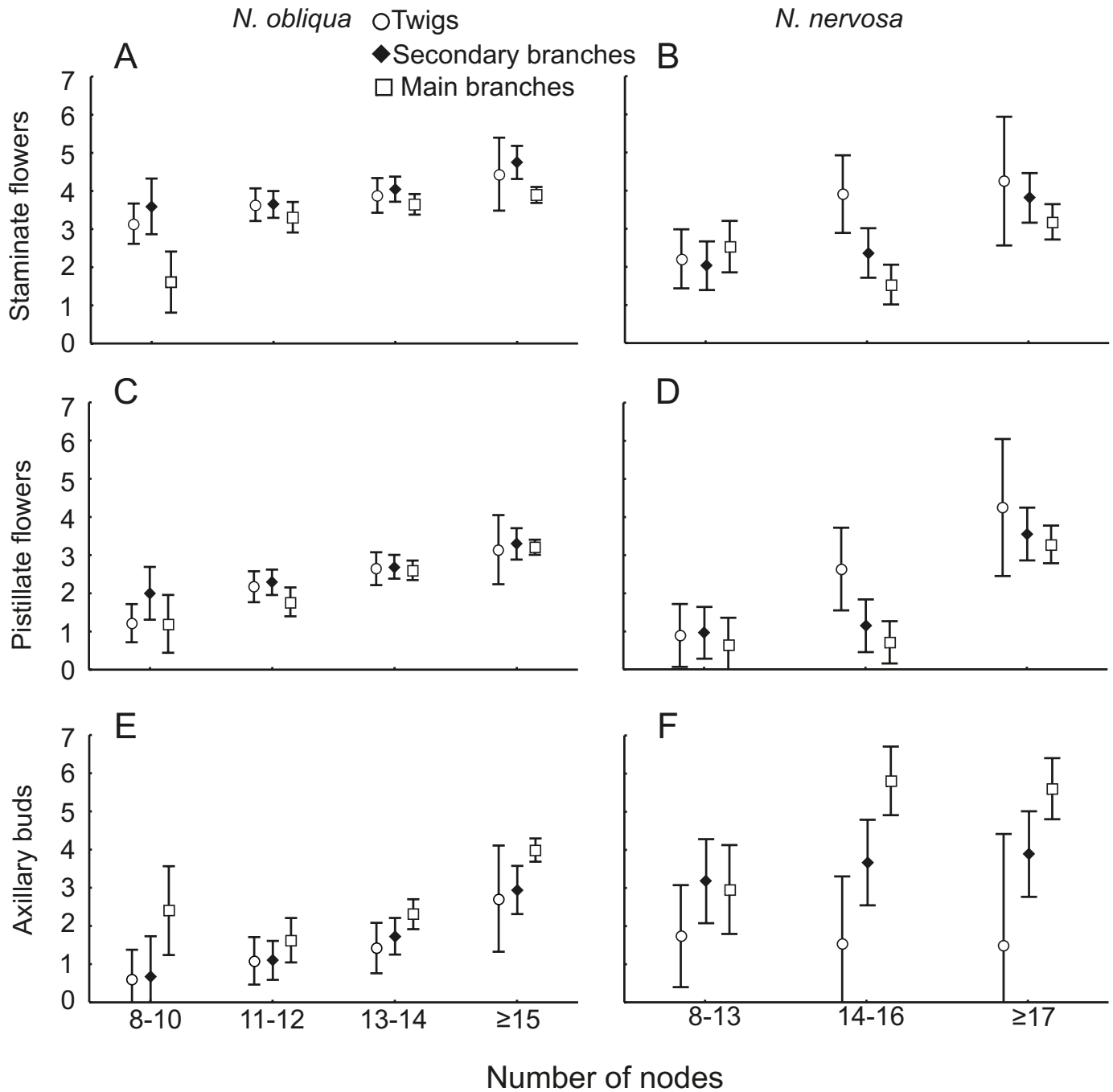
Distributions of flowers and flowering shoots

For *N. obliqua* and *N. nervosa*, the three topologically defined axis categories considered in this study are clearly different regarding their vegetative traits (Table 1). On the other hand, these axis categories have similar proportions of bisexual, staminate, and pistillate shoots, implying that qualitative flower production does not differ among axis categories (Figs. 3A, 3B). Shoot sexuality was related to shoot size in *N. nervosa*; staminate shoots were more common among short and intermediate-size shoots, and bisexual shoots more common among long shoots (Fig. 3D). In other words, long flowering shoots of *N. nervosa* are more likely to bear pistil-

late flowers than short and intermediate-size flowering shoots. The more distal position of pistillate flowers in shoots of *N. nervosa* than in those of *N. obliqua* (Puntieri et al. 2009) indicates that more nodes must be developed in the former than in the latter species to initiate pistillate flowers. Nonetheless, the high variations in shoot size within each axis category meant that staminate, pistillate, and bisexual shoots were found on any of the three axis categories considered here.

Like in *Nothofagus*, species of the closely related Fagaceae family have staminate and pistillate flowers on the same shoots, in the same or in separate inflorescences (Kaul 1986; Nicolini 1997; Gleissner 1999). We are aware of no studies evaluating quantitative or qualitative variations in shoot sexuality among axis categories in species of Fagaceae (but see Comps et al. 1994). In other related species, like those of *Betula* and *Alnus* (Betulaceae), staminate and pistillate flowers are developed on different shoots (Ishihara and Kikuzawa 2009). At least in some species of *Betula*, the production of

Fig. 5. Mean and 95% confidence interval for the numbers of staminate flowers (A, B), pistillate flowers (C, D), and axillary buds (E, F) for sibling shoots of *Nothofagus obliqua* and *N. nervosa* of different size classes and derived from three axis categories (MB, main branches; SB, secondary branches; TW, twigs).



pistillate or staminate shoots depends on the axis concerned (Ishihara and Kikuzawa 2004), which suggests that reproductive differentiation among axes and the distribution patterns of the flowers over the crown are more notable in this genus than in *Nothofagus*.

Numbers of flowers as related to shoot size and axis category

In the present survey, quantitative flower production for *N. obliqua* and *N. nervosa* was compared among axis categories and shoot sizes at two levels; single shoots (here referred

to as sibling-shoot level) and all shoots derived from a previous-year shoot (parent-shoot level). The number of pistillate flowers per sibling shoot increased proportionally more than the number of nodes of the sibling shoots (Table 2). The number of staminate flowers did not vary with sibling-shoot size in the case of *N. nervosa* and increased proportionally less than the number of nodes of the sibling shoots in *N. obliqua* (Table 2). This implies that in reproductive shoots of these species the shoot section from which staminate flowers may develop is more precisely delimited than that from which pistillate flowers may develop, in agreement

Table 3. Statistical comparisons of the numbers of flowers among axis categories at the sibling-shoot level.

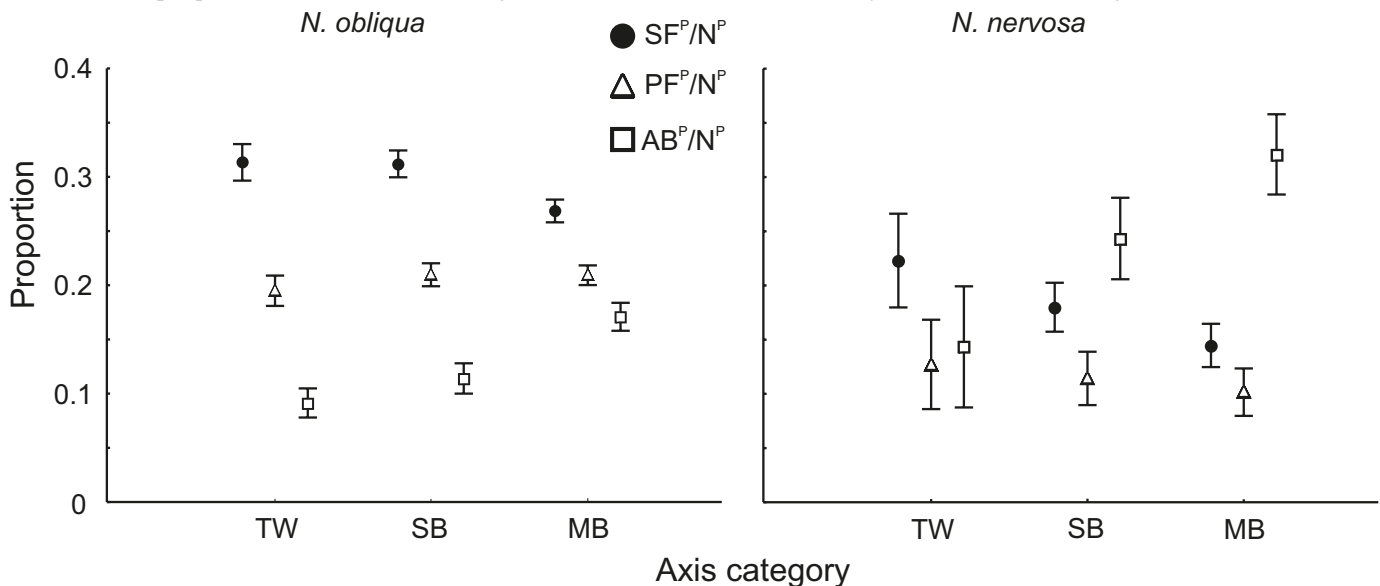
	Axis category		Individual		No. of nodes		Axis category × individual		R^2
	F	p	F	p	F	p	F	p	
<i>Nothofagus obliqua</i>									
SF ^S ($n = 590$)	1.86	0.167	25.31	<0.001	69.4	<0.001	2.31	<0.001	72.5
PF ^S ($n = 563$)	1.36	0.267	5.58	<0.001	58.03	<0.001	1.62	0.008	51.1
<i>Nothofagus nervosa</i>									
Log (SF ^S) ($n = 199$)	2.18	0.116	10.09	<0.001	0.23	0.631	—	—	49.5
Log (PF ^S) ($n = 140$)	0.72	0.987	5.62	<0.001	33.24	<0.001	—	—	61.1

Note: Results of two-way ANCOVA assessing the effects of axis category (fixed factor), individual tree (random factor), number of nodes per sibling shoot (covariable), and the axis category × individual tree interaction on flower production. SF^S, number of staminate flowers per sibling shoot; PF^S, number of pistillate flowers per sibling shoot. R^2 (%), coefficient of determination associated with the ANCOVA. Other details like in Table 1.

Table 4. Statistical comparisons of the numbers of flowers among axis categories at the parent-shoot level.

	Axis category		Individual		Log (N ^P)		R^2
	F	p	F	p	F	p	
<i>Nothofagus obliqua</i> ($n = 75$)							
Log (SF ^P)	1.27	0.289	1.40	0.157	214.3	<0.001	95.3
Log (PF ^P)	0.98	0.381	1.04	0.440	131.1	<0.001	94.8
<i>Nothofagus nervosa</i> ($n = 44$)							
Log (SF ^P)	1.70	0.190	1.81	0.092	41.6	<0.001	87.0
Log (PF ^P)	0.95	0.398	2.96	0.008	10.9	0.003	79.9

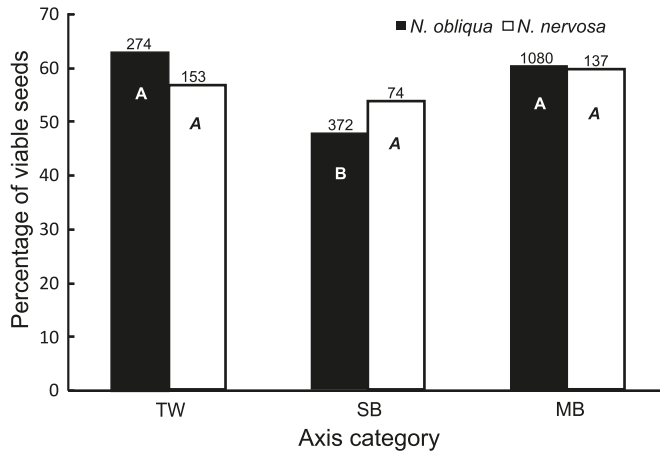
Note: Results of mixed ANCOVA assessing the effects of axis category (fixed factor), individual tree (random factor), and the number of nodes per parent shoot (N^P, covariable). SF^P, total number of staminate flowers derived from parent shoots; PF^P, total number of pistillate flowers derived from parent shoots (see Fig. 2). R^2 (%), coefficient of determination associated with the ANCOVA. Other details like in Table 1.

Fig. 6. Mean and 95% confidence interval for the relative numbers of staminate flowers (SF^P/N^P), pistillate flowers (PF^P/N^P), and axillary buds (AB^P/N^P) per parent shoot for three axis categories: main branches (MB), secondary branches (SB), and twigs (TW).

with previous results (Puntieri et al. 2009). As a consequence, the longer a flowering shoot, the more prevalent its female function. The tendency of *Nothofagus* species to develop longer sibling shoots from distal rather than proximal nodes of parent shoots (Puntieri et al. 2003) suggests that

pistillate flowers would be more concentrated in shoots located at distal than at proximal positions on the parent shoot (as found also for *Quercus*; Kaul 1986). However, the number of pistillate flowers per sibling shoot was similar for axis categories located at different positions within the tree crown.

Fig. 7. Percentage of viable seeds after open pollination for main branches (MB), secondary branches (SB), and twigs (TW) of *Nothofagus obliqua* (black bars) and *N. nervosa* (white bars). The numbers of dissected seeds are indicated at the top of the bars. Different letters indicate significant differences among axis categories for each species (χ^2 tests).



Therefore the idea, stated in the introduction, that pistillate flowers would be more concentrated at the crown's periphery was not supported (Table 3).

The analysis of flower production at the parent-shoot level found that, in both species, the production of staminate flowers increased proportionally less than the sum of the numbers of nodes of all sibling shoots (Table 2). The same could be said for the number of pistillate flowers of *N. nervosa*. For parent shoots of *N. obliqua*, in contrast, the increase in the number of pistillate flowers was proportional to that in the number of nodes of all sibling shoots. For these species, meristematic availability relates positively with the added number of flowers of all sibling shoots, as shown for other species (Lehtilä and Larsson 2005), although the production of staminate flowers in both *Nothofagus* species and that of pistillate flowers in *N. nervosa*, would become proportionally less important as the number of nodes derived from a parent shoot increases. Similarly to what was observed for single sibling shoots, the axis category of a parent shoot did not affect its flower production (Table 4).

Taking all our results together, we may conclude that in *N. nervosa* and *N. obliqua* flower production is determined mainly by shoot size and not by the axis category of the shoot as defined by its topological position in the crown. Shoot size is related to the proportional assignment of meristems to staminate and pistillate flowers, and this is more notable at the sibling-shoot than at the parent-shoot level. It is worth noticing that the individual tree contributed significantly to the variability in the number of pistillate flowers per parent shoot in the case of *N. nervosa* (Table 4). The possible involvement of genetic (including ontogenetic) and (or) environmental factors on flower production should be investigated for both species in even-aged populations of known genetic origin.

Vegetative and reproductive roles in *Nothofagus* axes

Flower production in trees involves the development of axes and (or) shoots with different degrees of structural specialization depending on the species (Bell 1991; Suzuki

2000, 2001; Normand et al. 2009). By concentrating flower production on highly specialized shoots, trees may separate vegetative and reproductive functions in space and time (e.g., in *Betula* and *Alnus*; Caesar and Macdonald 1983; Ishihara and Kikuzawa 2009), and concentrate reproductive demands of resources and meristems. In *Nothofagus*, flowers develop on leafy shoots that play both vegetative and reproductive roles (as in *Fagus*; Comps et al. 1994). In these trees, flowers develop simultaneously with leaves as shoots extend, and, according to our results, the number of reproductive organs is positively related to the number of nodes (cataphylls + green leaves) per flowering shoot, like in other tree species (Normand et al. 2009; Sánchez-Humanes et al. 2011). Inflorescences in *Nothofagus* are so small that their interference with vegetative functions in terms of resource allocation may seem irrelevant, at least before the development of fruits and seeds. The present study indicates that flower production has a cost in terms of axillary buds and, therefore, future branching in the three axis categories considered. Since pistillate and staminate flowers have different positions along shoots (Puntieri et al. 2009), their respective impacts on branching would be rather different. Pistillate flowers occupy intermediate nodes from which branches develop 1 year after shoot extension in nonflowering shoots. Therefore, the development of pistillate flowers affects branching in the year following that of flower production. The cost of staminate flowers may be evident in the longer term, as these flowers occupy the nodes from which branches could have developed several or many years after parent-shoot extension (Barthélémy et al. 1999). Based on our results, the most severe restriction to future growth imposed by flowering may be found in short flowering shoots, in which few or no vegetative meristems remain after flowering (see Figs. 5E, 5F, and 6). On the contrary, long flowering shoots have proportionally higher productions of axillary buds and, consequently, a higher branching potential than short shoots.

Anemophilous tree species usually have massive flower production in compensation for the significant influence of random factors on pollination. In these species, the location of flowers at the periphery of the crown would favor cross-pollination (Lovett Doust and Lovett Doust 1988; Ipinza and Espejo 2000). Compared with other anemophilous trees, *Nothofagus* trees would have low pollination efficiency owing to their small inflorescences. In addition, the simultaneous occurrences of anthesis and leaf expansion would mean the obstruction of pollen flow by leaves. This seems to be compensated with the production of flowers on shoots of variable sizes and axis categories, as observed in this study, with a reduction in branching and leaf development in the following year. Thus, flower production would also be curtailed 1 year after massive flowering, as observed for *Nothofagus* in previous studies (Donoso et al. 2006a, 2006b). Tree species in which massive flowering occurs must have evolved regulation systems to balance vegetative and reproductive functions (Obeso 2002), and the effect of flowering on the distribution of meristems for future growth may be involved in such a regulation.

Seed viability and axis category

This study indicates that, at least for *N. obliqua*, the probability of setting viable seeds on a flowering shoot depends

on the axis from which the shoot derived. In the introduction, we proposed that shoots at the crown periphery would be more likely to set viable seeds owing to the higher likelihood of alien pollen reaching these shoots. This idea is not supported by the results. Shoots arising from main branches (the most peripheral axes) and twigs (the least peripheral ones) developed proportionally more viable seeds than those derived from secondary branches. The fact that the two topologically and morphologically most contrasting axes analysed here (main branches and twigs) produced similar percentages of viable seeds, suggests that seed development in shoots of this species would not be affected by functional aspects of the flowering axes. Moreover, the idea that resource limitation is not an important determinant of seed viability in this species would be supported by the fact that nearly all pistillate flowers developed into fruits (though most of them devoid of viable seeds; Riveros et al. 1995a).

Based on our results, the lower percentage of viable seeds in secondary branches of *N. obliqua* may be explained by alluding to pollination conditions. In *N. obliqua* and *N. nervosa*, pollen release occurs during the expansion of leaves, which could be a physical barrier to pollen flux. This interference could be maximal at intermediate crown zones where secondary branches are positioned. Closer to the trunk, the interference to pollen flux generated by leaves would be lower since twigs consist of shoots with less leaves and less branches (owing to high meristem expenditure in flower production). In addition, differences between secondary branches and twigs regarding the numbers of leaves and branches and, therefore, staminate flowers, would imply higher probabilities of self-pollination (ineffective in these species) in the former than in the latter axis categories. Pollen flux has been assessed at individual and population scales (Riveros et al. 1995b; Knapp et al. 2001), but so far there are, to our knowledge, no studies that quantify pollen flux at different positions within the crown. In the case of *N. nervosa*, the statistical difference in seed viability among axes categories did not reach a significant level, but a trend similar to that obtained in *N. obliqua* was observed (Fig. 7).

Conclusions

In *N. obliqua* and *N. nervosa*, flowering shoots are developed on main branches, secondary branches, and twigs, and most of them bear both flower types. In the case of *N. nervosa*, the majority of flowering shoots bearing only staminate flowers are short and intermediate sized, whereas most hermaphrodite flowering shoots are long. Quantitative differences in flower production among axis categories are mainly related to differences in the number and size of the sibling shoots produced by each axis category. Flowering shoots arising from main branches produce proportionally more axillary buds than those arising from secondary branches and twigs. The likelihood of seed set in *Nothofagus* seems to be lower in secondary branches than in main branches and twigs.

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