Preformation and distribution of staminate and pistillate flowers in growth units of Nothofagus alpina and N. obliqua (Nothofagaceae)

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INTRODUCTION

Reproductive characters that determine mating are highly influential in plant macro-evolution (Barrett et al., 1997). In the case of plant species with hermaphrodite, insect-pollinated flowers, the sizes, relative positions and maturing times of stamens and carpels are among the reproductive characters that have been studied (e.g. Barrett et al., 1997; Gerber and Moeller, 2006; Bolmgren and Cowan, 2008). The equivalent reproductive characters in the case of plant species with unisexual (i.e. dichogamous) wind-pollinated flowers are the distributions and times of differentiation and anthesis of staminate and pistillate flowers (e.g. Kaul and Abbe, 1984; Kaul, 1986; Friedman and Barrett, 2008). Among the major plant clades with unisexual wind-pollinated flowers is that including the tree families Fagaceae, Nothofagaceae and Betulaceae (order Fagales; APG II, 2003), where staminate and pistillate flowers are developed in simple or complex inflorescences (Kaul and Abbe, 1984). For these families, the distribution and differentiation times of flowers have been investigated only from a qualitative perspective, which has proved useful for comparing genera or families (e.g. Kaul and Abbe, 1984; Yacine and Bouras, 1997). Comparisons of these traits between species belonging to the same genus may demand more detailed, quantitative analyses. To our knowledge, such analyses have seldom been attempted so far (but see Ishihara and Kikuzawa, 2004). Even at the single-species level, within this plant clade few studies have dealt with the times of flower differentiation and extension in relation to the development of vegetative organs (e.g. Caesar and Macdonald, 1983).

Since the end of the 1990s, hidden Markov chain models have greatly improved the characterization of the distribution of axillary productions, including flowers (see Guédon et al., 2001 for a review; for the mathematical specification of a model see Guédon, 2003, 2005; for specific applications on plants see Costes and Guédon, 1997, 2002; Grosfeld, 2002; Heuret, 2002; Seleznyova et al., 2002; Costes et al., 2003; Guédon et al., 2003, 2007; Heuret et al., 2003; Renton et al., 2006; Louarn et al., 2007; Leroy and Heuret, 2008). These models are relevant in order to (1) quantify and compare, within and between species, the distribution of axillary organs along growth units (GU: defined as a stem portion derived from a single, uninterrupted extension event); (2) reveal, based on these distributions, homogeneous segments of GUs not easily identifiable by simple observation; and (3) describe the distributions of these segments and the transitions between them.

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The deciduous tree species *Nothofagus alpina* (= *N. nervosa*) and *N. obliqua* grow naturally in Chile and Argentina (*N. alpina*: 35°13’S to 41°15’S; *N. obliqua*: 30°30’S to 40°15’S; Donoso et al., 2007a, b). They are most valued in terms of timber production due to their growth rate and wood quality (Stewart, 1979; Destremau, 1988; Deans et al., 1992). In young trees of *N. alpina* and *N. obliqua*, each axis is constituted by a sequence of GUs, each of which extends in the spring–summer period (Puntieri et al., 2006, 2007a, b). For *N. alpina*, it has been shown that most GUs consist entirely of organs differentiated in the previous growing season (preformed organs) whereas the largest GUs include, in addition to the preformed organs, a distal set of organs whose differentiation and extension are simultaneous (neoformed organs; Guédon et al., 2006; Puntieri et al., 2007b). Flowering GUs, i.e. those on which at least one flower has developed, have not been included in these studies.

The distribution and time of differentiation of flowers in the genus *Nothofagus* has not been considered in depth. Published descriptions refer to small, axillary 1- to 3-flowered staminate and pistillate inflorescences (Hoffmann, 1982; Correa, 1984) and to anthesis times occurring at the time of extension of the flowering GUs (Riveros et al., 1995; Barthélémé et al., 1999; Ipinza and Espejo, 2000; Rozefelds and Drinnan, 2002). The presence of primordia of staminate flowers in winter buds has been mentioned (Ipinza and Espejo, 2000) but no reports indicate whether all staminate and pistillate flowers of a GU are preformed.

The goals of this study were to: (1) analyse and compare the distributions of flowers along GUs; (2) determine the preformed and/or neoformed nature of flowering GUs; and (3) elaborate Markovian models describing the distributions of staminate and pistillate flowers along GUs of *N. alpina* and *N. obliqua*.

**MATERIALS AND METHODS**

**Sampling site and GUs**

Sampling was performed at Lanín National Park, Argentina, between 40°16’ and 40°09’S and between 71°36’ and 71°38’W. The natural vegetation in this area includes mixed forests of *Nothofagus alpina*, *N. obliqua* and *N. dombei*. GUs and buds were sampled from trees of different ages and from diverse habitats: forest clearings, road margins, and urban/suburban areas. These trees were between 10 and 35 m high and between 25 and 120 years old.

The extents of preformation and neoformation were assessed by means of two samples taken at the end of successive growing seasons, in winter (September) 2005 and autumn (April) 2006. Each sample consisted of a set of GUs extended in the 2004–2005 growing season (henceforth referred to as parent GUs) and all the organs derived from them. GUs were taken from main branches derived at ≤5 m high from the trunk of adult trees. The main branches selected were those in which scars left by flowers, fruits and/or fruit cupules could be seen on the stem (Fig. 1–C). In order to have a number of GUs high enough for mathematical modelling, and due to the small number of flower-producing trees, no restriction was imposed in terms of main-branch size.

![Fig. 1](image-url) Morphology of growth units (GUs) and buds of *Nothofagus alpina*. (A) Proximal end of a GU in which the scars left by an abscised staminate flower (sfs) and its subtending leaf (ls) are indicated. (B) Axis consisting of two successive flowering GUs (the limit between them is indicated by a double line); standing fruit cupules are shown. (C) Scar on the stem after the abscission of a fruit cupule (cs). (D) Bud with primordia of staminate flowers (sf) after partial dissection. (E) Distal GU in which axillary buds are evident; the one indicated with an arrow included flower primordia. (F) Vegetative bud (similar to that on the right of D). (G) Axillary bud after dissection; the bud’s apex and the scar left on the stem by a removed stipule (sts) are indicated. (H) Detail of the primordium of a triad of pistillate flowers (pf). Several GUs were sampled from each selected tree and some trees were included in both samples.

In winter 2005, 93 parent GUs from 32 *N. alpina* trees and 63 parent GUs from 38 *N. obliqua* trees were sampled (sample 1). At that time, each parent GU had axillary buds at some of its nodes (all terminal buds had died after parent GU extension, a common trait in adult *Nothofagus* trees; Barthélémé et al., 1999; Stecconi, 2006). The nodes of each parent GU were numbered following a proximal-to-distal ranking, and the types of leaf (cataphyll or green leaf) and axillary organ (none, bud, staminate flowers and pistillate flowers) at each node were recorded. In *Nothofagus*, staminate and pistillate flowers develop in inflorescences with one-to-three tightly arranged flowers (Hoffmann, 1982; Correa, 1984). Each inflorescence was counted as a single flower in the present study. Since leaves and flowers had already abscised by the time of sampling, their development at each node of a parent GU was identified through the observation of scars on the stem, as in previous studies (Barthélémé et al., 1999; Puntieri et al., 1999; Fig. 1). For each GU, all buds longer than 2 mm were dissected under a stereomicroscope (Olympus SZH10, 70×) after submerging the GUs in 70% ethanol for 2 weeks. The types of leaves (cataphylls and green leaves) and axillary organs (none, bud, primordia of staminate flowers and primordia of...
pistillate flowers) were determined for each node counted from the proximal end of each bud. In some cases a positive identification of the most distal axillary organ was not possible due to its small size.

In autumn 2006, 65 parent GUs from 28 *N. alpina* trees and 57 parent GUs from 36 *N. obliqua* trees were sampled (sample 2). At this stage, each parent GU present in winter 2005 had branches extended in the 2005–2006 growing season. Nodes of each parent GU were numbered and their types of leaves and axillary structures (none, bud, staminate flower, pistillate flower and branch) identified. For each branch, the length and number of nodes and the types of leaf and axillary organ at each node were recorded. For each parent GU, the sum of all nodes produced by all of its branches was calculated. Whenever the identification of an axillary organ was doubtful (mainly due to the damage caused by insects at the node), such an organ was considered as unidentified.

**Comparisons between parent GUs and between buds and branches**

For each species, parent GUs were compared between samples (with Mann–Whitney *U*-tests; Sokal and Rohlf, 1981). Attributes quantified were the numbers of nodes, cataphylls, green leaves, nodes without morphologically visible axillary production, nodes with buds or branches, and number of flower-bearing nodes in flowering GUs. The percentages of flowering parent GUs with staminate flowers, pistillate flowers and both flower types were compared between samples by means of  \( \chi^2 \) tests.

To quantify the extent of preformation, the numbers of nodes of sample 1 buds were compared with the numbers of nodes of sample 2 branches using ANCOVA (Sokal and Rohlf, 1981). In this comparison, sample number was included as a fixed factor. The increasing proximal-to-distal gradient in the size of buds and branches, characteristic of branching systems in *Nothofagus* (Puntieri et al., 2003), was accounted for by including the ordinal number of the parent-GU node corresponding to each bud or branch as a covariable.

In order to assess the effect of flowering on the further branching of a GU, we computed, for each species, the product-moment correlation coefficient (r; Sokal and Rohlf, 1981) between the sum of the nodes of all branches derived from sample 2 parent GUs and the numbers of staminate and pistillate flowers developed on the parent GU.

**Modelling axillary productions with hidden semi-Markov chains**

Modelling was applied for GUs sampled in winter (sample 1) and the following autumn (sample 2) in order to identify and describe segments with homogenous axillary production and the transitions between these segments. For each parent GU, a sequence of axillary productions (nodes ranked from base to top on parent GU) was prepared. For each node, the type of axillary production was recorded as: no axillary production observable, bud, staminate flower, pistillate flower, branch and unidentified organ. Total numbers of sequences of axillary structures were 158 for *N. alpina* (93 for sample 1 and 65 for sample 2) and 120 for *N. obliqua* (63 for sample 1 and 57 for sample 2). Henceforth, each kind of axillary production is termed an ‘event’ (Guédon et al., 2001; Heuret et al., 2003). The first step in the modelling of these sequences was an exploratory analysis of the sequence structure (for more details see Guédon et al., 2001; Heuret et al., 2003). We focused on the frequency distribution of each event as a function of the node rank.

The statistical model used was a hidden semi-Markov chain estimated using the AMAPmod software (Godin et al., 1997). In this analysis, sequences of events are viewed as successions of homogeneous segments. Composition properties, in terms of type(s) of event(s), do not change substantially within each segment but do change between segments. The first step in model generation is the description of the succession of segments and the length of each segment in terms of number of nodes. In mathematical terms, these segments represent states that are connected by transitions; the sum of all probabilities of transitions from a given state to any of the others (e.g. from state *i* at rank *n* – 1 to state *i* at rank *n*) equals one. Initial probabilities are also needed to select the initial state. The occupancy distribution, represented by the length of the segment, is computed for each state except for the final absorbent state. The final absorbent state is defined as the one that once entered cannot be quitted, and is necessary for the mathematical specification of the model (Guédon, 2003). The time spent in this state (which is infinite) cannot be represented by an explicit state occupancy distribution. In our study, the final absorbent state was artificially added at the end of the sequences and did not correspond to botanical data. This was performed in order to estimate the length of the most distal segment. Each transitory state was associated with a discrete distribution representing the events observed within the segment.

The maximum likelihood estimation of parameters of a hidden semi-Markov chain requires iterative optimization techniques that are applications of the expectation–maximization (EM) algorithm. For the biological interpretation of the statistical modelling it is useful to determine the optimal segmentation of the observed sequences. This optimal segmentation is obtained by using a dynamic programming method usually referred to as the Viterbi algorithm (Guédon, 2003, 2005). In order to represent graphically the estimated hidden semi-Markov chains the same convention proposed in Guédon et al. (2001) was used.

Mann–Whitney *U*-tests (Sokal and Rohlf, 1981) were used to compare the lengths of the segments estimated in the models on the basis of the subjacent distributions issued from optimal segmentation.

**RESULTS**

**Size and axillary productions of parent GUs**

For each species, the numbers of nodes, cataphylls and green leaves of parent GUs were similar between samples 1 and 2 (Table 1). Parent GUs of both species had about six basal cataphylls. The numbers of green leaves per GU varied between four and 20 for *N. alpina* and between four and 26 for *N. obliqua*, and both species had between five and six proximal nodes per GU in which no axillary organs were observed. For both species, the number of axillary buds of sample 1 GUs was higher than the number of branches of sample 2 GUs (Table 1).
Between 75 and 80 % of *N. alpina* GUs and about 60 % of *N. obliqua* GUs had developed at least one flower, either pistillate or staminate, at the time of their extension; no differences were detected between samples in this regard (Table 1). The majority of flowering parent GUs of both species had developed staminate flowers and pistillate flowers. The maximum numbers of staminate flowers per GU (irrespective of whether pistillate flowers were present or not) were eight for *N. alpina* and 12 for *N. obliqua*; in both species up to nine pistillate flowers per GU were found. When adding up staminate flowers and pistillate flowers, the maximum numbers were 13 for *N. alpina* and 17 for *N. obliqua*. The percentages of parent GUs with only staminate flowers were similar between samples for both species. Similar percentages of parent GUs of samples 1 and 2 had developed only pistillate flowers in the case of *N. obliqua*. For *N. alpina* a lower percentage of GUs with only pistillate flowers was found for sample 2 than for sample 1 (Table 1). Differences between samples concerning the number of flowering GUs were not significant except in the case of *N. alpina* GUs with only pistillate flowers: those of sample 1 had more nodes than those of sample 2.

The distributions of axillary organs on parent GUs were, in general terms, similar for both samples (Fig. 2). As the frequency of nodes without morphologically visible auxiliary structures decreased, the frequency of nodes with staminate flowers increased, reaching its highest values between nodes six and ten for both species. For *N. alpina* the development of axillary buds on parent GUs of sample 1 reached the highest frequency for nodes nine to 12 and declined to a frequency of about 0.5 for more distal nodes (Fig. 2A). For sample 2 of this species the frequency of nodes with branches increased for intermediate positions and levelled off for distal positions (Fig. 2B). In the case of *N. obliqua*, the proportion of bud-bearing nodes of sample 1 parent GUs increased gradually from proximal to distal positions (Fig. 2C). Parent GUs of *N. obliqua* from sample 2 had a low frequency of axillary buds between nodes five and ten, whereas the frequency of axillary branches increased from intermediate to distal nodes (Fig. 2D). For both species, the proportion of nodes with pistillate flowers increased at intermediate positions and tended to stabilize or decrease (more notably so in the case of *N. obliqua*) towards the distal end of GUs (Fig. 2).

The sum of the nodes of all branches derived from sample 2 parent GUs averaged 66 nodes (s.e. = 6.3) for *N. obliqua* and 39 nodes (s.e. = 2.9) for *N. alpina*. These sums were negatively correlated with the corresponding numbers of pistillate flowers per parent GU, both for *N. alpina* (r = -0.41; P < 0.001) and *N. obliqua* (r = -0.27; P < 0.05). The sum of the nodes of all branches of sample 2 parent GUs was negatively correlated with the number of staminate flowers per parent GU for *N. alpina* (r = -0.30; P < 0.05) but not for *N. obliqua* (r = -0.15; P > 0.05).

### Models of axillary production for parent GUs

**Nothofagus alpina**. For parent GUs of sample 1, the model estimated from the sequences consists of six transient states (A–F; Fig. 3). State A corresponds to the proximal segment (mean = 5.8 nodes) devoid of visible axillary production and is...
followed either by states B and C, or by state D. State B corresponds to a segment (2-1 nodes) associated to staminate flowers, while states C and D have bud-bearing nodes. The sequence formed by states B and C has slightly less nodes (6.6) than state D (7.8; \( P \), 0.05). States C and D are followed by state E (3.3 nodes), associated to pistillate flowers, which is followed either by state F, defined by a short segment of buds (1-9 nodes), or by the absorbent state G. State F is also accessible from state C.

For parent GUs of sample 2, the model generated has seven transient states (A–G; Fig. 3). State A corresponds to a proximal segment of 6-4 nodes devoid of visible axillary organs and can be followed directly by state D, defined by three buds, or by states B and C, both with staminate flowers. States B and C correspond to segments that differ in their number of nodes (2.3 and 7.5 nodes, respectively). State E corresponds to a segment of about three nodes with branches (73 %), unidentified structures (21 %) and buds (5 %). State E is accessible from states B and D and can be followed by state F (3-4 nodes), with pistillate flowers, or by state G (1-4 nodes), with branches. State F can be the most distal state of the GU, but in most cases it is followed by state G.

States A, B, E and F in the model estimated from sample 1 are equivalent, respectively, to states A, B, F and G in the model estimated from sample 2. No significant differences in the number of nodes of equivalent states were found between samples (\( P > 0.05 \)) except for state A (longer for sample 2, 6-4 nodes, than for sample 1, 5-8 nodes; \( P < 0.01 \)). Another difference between both models was caused by state C of sample 2, which resulted from the occurrence of four GUs with relatively long sequences of staminate and pistillate flowers. States C and D in the model estimated for sample 1 resembled states D and E in the model of sample 2. The number of nodes before the first occurrence of pistillate flowers was similar for samples 1 and 2 (7-9 and 8-3 nodes, respectively; \( P > 0.05 \)).

Nothofagus obliqua. The models estimated for parent GUs of samples 1 and 2 consist of five transient states (A–E; Fig. 4). State A corresponds to a proximal segment devoid of axillary organs (5-6 nodes for sample 1 and 5-3 nodes for sample 2), and is followed by state B (4-1 nodes for sample 1 and 4-9 nodes for sample 2), associated either to staminate flowers (81 % and 87 % for samples 1 and 2, respectively) or buds (19 % and 12 %). State B is, in most cases, followed by state C that corresponds to a segment with pistillate flowers (2-3 nodes for sample 1 and 3-8 nodes for sample 2) and, sometimes in the case of sample 2, with branches (8 %). State C is always followed by a segment of buds (in the case of sample 1) or branches (for sample 2), which may be either long (8-2 and 8-8 nodes for samples 1 and 2, respectively; state D), or short (2-4 and 2-8 nodes; state E). Transition probabilities from state B to states D or E were low.

The models estimated for samples 1 and 2 are similar in terms of number of states, occupancy distributions and transition probabilities. The number of nodes of the pistillate segment is
significantly higher for the model of sample 2 than for that of sample 1 ($P < 0.001$). The number of nodes before the first occurrence of staminate flowers or pistillate flowers is similar in the models of samples 1 and 2 (respectively 5.5 and 5.2 nodes for staminate flowers, $P > 0.1$; 9.6 and 9.5 nodes for pistillate flowers, $P > 0.1$). The number of nodes before the first occurrence of a bud in the model of sample 1 (10.0 nodes) is significantly lower than the number of nodes before the first branches in the model of sample 2 (12.4 nodes, $P = 0.001$).

**FIG. 3.** Hidden semi-Markov chains estimated for the axillary productions of *Nothofagus alpina* GUs extended in the 2004–2005 growing season and sampled in winter 2005 and autumn 2006. Each state of the model (encircled letters: A–H) has one to several axillary productions (N: none, B: bud, S: staminate flower, P: pistillate flower, Br: branch, U: unidentified organ), whose proportions are indicated; X is an artificial absorbent state (see text for details). The theoretical distribution of each state’s occupancy distribution (expressed as number of nodes) is shown graphically. Transition probabilities between states are indicated (number associated to each arrow). Only transition probabilities $>0.05$ are represented.
Buds and branches

The number of nodes of sample 1 buds was higher than that of sample 2 branches for both species ($F = 35.5$ for *N. alpina* and $F = 34.4$ for *N. obliqua*; $P < 0.001$). The number of nodes per branch and, especially, per bud increased following a proximal-to-distal gradient ($F = 67.9$ for *N. alpina* and $F = 131.8$ for *N. obliqua*; $P < 0.001$). The length of branches increased from proximal to distal positions on their parent GUs for both species (Fig. 5).

**DISCUSSION**

**Flower distribution on GUs**

In *Nothofagus alpina* and *N. obliqua*, flowering GUs may include staminate and/or pistillate flowers. The distributions
of these flowers along flowering GUs of these species follow precise patterns irrespective of whether one or both flower types are developed. Staminate flowers may derive from the sixth to the ninth proximal nodes of a GU, whereas pistillate flowers may derive from the ninth node onwards. Although in flowering GUs of both species there is a high probability (>70%) of staminate flowers developing after the ‘nude’ proximal-end of the GU, axillary buds (which grow into branches in the next growing season) may develop in the position of staminate flowers and be followed by pistillate flowers.

With regard to the distribution of pistillate flowers along GUs, Markovian models evidenced a notable difference between N. alpina and N. obliqua that is not obvious through the observation of the frequencies of flowers at different nodes (Fig. 2). For N. obliqua, the proximal segment with staminate flowers or axillary buds is followed either by a segment with pistillate flowers or a segment with axillary buds. Irrespective of whether pistillate flowers are developed or not, once a N. obliqua GU starts producing axillary buds, it does not revert to flower production. Instead, flowering GUs of N. alpina frequently have a distinct segment with axillary buds between staminate flowers and pistillate flowers. The segment with pistillate flowers in this species may reach the distal end of the GU, but it is most often followed distally by a segment with buds. This means that, during the differentiation of a flowering GU of N. obliqua with both flower types, there is one transition from reproductive to vegetative axillary primordia [i.e. from (staminate flowers–pistillate flowers) to (stems/leaves)]. In the case of N. alpina GUs, on the other hand, there are frequently two transitions from reproductive to vegetative axillary primordia, separated by a segment of reversion from vegetative to reproductive primordia (between staminate and pistillate flowers).

Other South American Nothofagus species studied in this regard exhibit a flower distribution pattern similar to that described here for N. obliqua (see Barthélemy et al., 1999; Stecconi, 2006), which also resembled the flower distribution pattern observed for Fagus sylvatica (Nicolini, 1997). Further studies concerning flower distribution in a broad range of Fagus and Nothofagus species might help clarifying the primitive or advanced nature of the so-far unusual flower arrangement described here for N. alpina.

The differences in flower distribution along GUs between N. alpina and N. obliqua may have implications for the mating patterns of these allegedly wind-pollinated species (see Donoso, 1993; Ipina and Espejo, 2000). Due to the proximal-to-distal progress in the extension of organs of each GU, the development of staminate and pistillate flowers at different and specific positions within flowering GUs might result in different anthesis times for both flower types. The relationship between flower position on a GU and anthesis time has been suggested for other plants (Diggle, 1995), including species of Fagaceae with staminate and pistillate flowers on the same GU (Kaul and Abbe, 1984). If such a relationship also does occur in Nothofagus, a larger spatial gap between staminate and pistillate flowers along a GU would mean a lower probability of self-pollination at the GU level (which is highly ineffective for seed production in Nothofagus; Ipina and Espejo, 2000). Following this line of reasoning and considering the results of studies on other species (Anderson and Hill, 2002; Oddou-Muratorio et al., 2005), self-pollination would be less likely in N. alpina than in N. obliqua. Specific studies on the relationship between flower position and anthesis time at the GU level as well as the tree level are necessary to test these hypotheses. Such studies would also help in understanding the crossing system leading to the development of hybrids between these species.

The present study revealed a high degree of variation in the structure of flowering GUs of N. alpina and N. obliqua. Flowering GUs differed in the numbers of staminate and pistillate flowers and in the presence or absence of each of these two flower types. The sources of variation for the structure of flowering GU could be addressed by analysing branches of known architectural position on trees of known ages and ontogenetic growth stages.

Preformation of flowering GUs in N. alpina and N. obliqua

In the present study we evaluated the preformed or neo-formed character of flowering GUs of N. alpina and N. obliqua through comparing the composition of buds.
developed during one growing season with the composition of branches extended from these buds in the next growing season. Parent GUs of both samples were similar in terms of numbers of nodes, cataphylls and green leaves and the Markovian models describing the distribution patterns of axial organs were also similar between both samples of each species. Only minor differences between samples were found. Thus, we considered sample 1 buds and sample 2 branches to be comparable structures.

Dissected buds had more nodes than branches and the flowers could be observed through bud dissection, which indicates that these branches (including their flowers) may be described as entirely preformed. The death and abscission (for still unknown reasons) of the most distal primordia of buds during GU extension, also observed in previous studies on *Nothofagus* (Puntieri et al., 2000, 2002; Souza et al., 2000; García et al., 2006), would explain the higher number of nodes in buds than branches. This indicates that the maximum number of flowers to be developed from a GU in a given growing season would be determined at least one growing season before the anthesis of these flowers.

The extent of flowering of *N. obliqua* or *N. alpina* trees might be predictable from the dissection of buds under a stereomicroscope. Nonetheless, the estimation of the number of pistillate flowers per GU may be complicated by the smaller sizes of auxiliary primordia towards a bud’s distal end and the distal position of pistillate flowers. The effect of bud-digging insects also adds inaccuracy to the identification of the most-distal auxiliary structures of buds. Since pistillate flowers tend to be produced in more distal nodes for *N. alpina* than for *N. obliqua* (see below) this inaccuracy might be higher in *N. alpina*.

This study shows that the auxiliary buds on a flowering or non-flowering GU of *N. alpina* and *N. obliqua* were already initiated at the time their parent GUs were preformed in buds. But these auxiliary buds extend into branches at least two growing seasons after their inception (i.e. one growing season after parent GU extension). These results are similar to those of classical studies on some northern temperate species (Garrison, 1949a, b). The postponement in the extension of auxiliary buds relative to that of their parent GUs is a common feature in *Nothofagus*. Immediate branches, i.e. those extending simultaneously with their parent GUs, are common in young *Nothofagus* trees (Barthélémy et al., 1999; Puntieri et al., 1998, 2000, 2002, 2007a, b; Souza et al., 2000) but were not observed in the present study. In plants of Fagaceae, Nothofagaceae and Betulaceae, both neof ormation and immediate branching are traits typical of GUs of the trunk or high, vigorous branches (Fontaine et al., 1999; Puntieri et al., 2000, 2002). Thus, the absence of these traits in the present study must be interpreted with caution as the sampling for the present study included only low, accessible branches.

**Effects of reproduction on further axis growth**

For *N. alpina* and *N. obliqua*, the development of flowers at a GU implies a reduction in the number of nodes available for the production of axillary buds and, as a consequence, for future axis branching. The number of nodes developed by a GU the year following GU flowering (measured by the preformation in its buds or the size of its branches) was more negatively correlated with the number of pistillate flowers than with the number of staminate flowers developed by the GU. This result may be explained by the specific positions at which each flower type develops and the variations in branch size along a parent GU: branches corresponding to the nodes where pistillate flowers develop are usually longer than those corresponding to the nodes where staminate flowers develop. The more negative effect of pistillate flowering on the further branch growth for *N. alpina* than for *N. obliqua* is related to the more distal position of pistillate flowers in the former than in the latter species. The alternation of years of high and low fruit production observed in *Nothofagus* spp. (Wardle, 1984; Marchelli and Gallo, 1999; Ipinza and Espledo, 2000; Kelly et al., 2001; Rosas et al., 2004) could, at least partly, relate to the restriction in the number of large branches from a GU as a result of the production of pistillate flowers during GU extension.

**Conclusions**

For *N. alpina* and *N. obliqua*, staminate and pistillate flowers are differentiated in the growing season preceding that of their antheses and at precise locations on their parent GUs. Along a GU, both flower types are more distant from each other in *N. alpina* than in *N. obliqua*. In order to disclose possible sources of variability in the structure of flowering GUs, further research should take account of endogenous factors including the ages and ontogenetic growth stages of trees and axes (see Barthélémy and Caraglio, 2007). A major point in need of investigation is the relationship between flower position on a GU and the time of anthesis.

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