

Multi-stemmed trees of *Nothofagus pumilio* second-growth forest in Patagonia are formed by highly related individuals

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- **Background and Aims** Multi-stemmed trees (tree clusters) in *Nothofagus pumilio*, a dominant tree species in Patagonia, are very uncommon and are restricted to the edge of second-growth forests following human-provoked fires. No vegetative reproduction has been reported so far. The genetic structure of multi-stemmed trees of this species was investigated and it was hypothesized that genets within a cluster were more closely related than average in the population.
- **Methods** Fifteen clusters (composed of at least three purported stems) and 15 single trees were sampled at the edge of a second-growth forest and genotyped using two amplified fragment length polymorphism (AFLP) primer pairs. We obtained 119 polymorphic markers that allowed clonality to be determined, together with sibship structure and relatedness among samples.
- **Key Results** Clonality was detected in seven clusters but all clusters had at least two different genotypes. Full sibs were found exclusively within clusters and in all clusters. Within a cluster, stems that were not identified as full sibs were often half sibs. Relatedness values for the full sibs and half sibs were higher than the theoretical values of 0.5 and 0.25 but the relatedness between clusters was very low.
- **Conclusions** Tree clusters that are merged at the edge of the second-growth forest of *N. pumilio* are composed of stems of the same genotype and of other genotypes that are highly related (but not always). It is suggested that this peculiar genetic structure results from a combination of several causes, including selection for merging of related individuals.

Key words: AFLP, multi-stemmed trees, Patagonia, post-fire regeneration, sibship structure.

INTRODUCTION

In some tree species, trees grow in merged clusters, forming multi-stemmed trees. Most often, this is due to several shoots growing from the same root system, either naturally, or, more frequently, as a consequence of sprouting after major disturbance events (fires, droughts, hurricanes; Bond and Midgley, 2001; Del Tredici, 2001). This pattern can also be observed at the upper forest edge in mountains or at the northern limit of arctic forests where trees lose their apical dominance and grow in creeping or multi-stemmed growth forms due to the combined effect of snow, wind and low temperatures ('krummholz'; Devi *et al.*, 2008; Wardle, 2008). In all these cases, the different stems are ramets of a single genet resulting from clonal growth. In other cases, however, multi-stemmed trees result from the merging of different individuals as is the case in some pine species of the subgenus *Strobus*. In limber pine (*Pinus flexilis*) (Linhart and Tomback, 1985; Schuster and Mitton, 1991; Carsey and Tomback, 1994), whitebark pine (*P. albicaulis*) (Linhart and Tomback, 1985; Furnier *et al.*, 1987) and Swiss stone pine (*P. cembra*) (Tomback *et al.*, 1993), the merged stems correspond to different individuals, which are on average more related to each other than random individuals from the population. Over an entire stand of *P. albicaulis*, however, individuals in

neighbouring clusters are not more similar than individuals in distant clusters (Furnier *et al.*, 1987); consequently, the distribution of genotypes across the population is likely to be random (Jorgensen and Hamrick, 1997). This is contrary to species with wind-dispersed seeds, which typically display associations between the degree of relatedness and distance from a seed source (Hamrick and Nason, 1996). The seeds of all these pine species are dispersed specifically by Clark's (*Nucifraga columbiana*) and spotted (*Nucifraga caryocatactes*) nutcrackers (for *P. albicaulis* and *P. flexilis* or *P. cembra*, respectively), that bury seeds in caches for winter provision. More than 80 % of the seeds are retrieved, but the few remaining seeds germinate and grow into clusters of merged trees. The high relatedness in these clusters indicates that several seeds in a cache are related as half to full siblings (Carsey and Tomback, 1994). Merging was also found in six species of strangler fig trees resulting in single-stem hollow trees (Thomson *et al.*, 1991) with branches differing by only a few loci (one to four isozyme loci out of 18 systems). For tree species with wind-dispersed seeds the genetic composition of merged tree clusters and the relatedness among the genets is largely unknown.

Strong positive interactions among individuals, such as merging, can be interpreted as a form of cooperation. In strangler figs, for example, the benefit for young seedlings to fuse is a gain