

Short Communication

Some wild bamboo clumps contain more than one genet

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Abstract. In clumping clonal plants, the often discrete nature of clumps may be enhanced by competition among genets. We examined the genetic composition of clumps of *Bambusa arnhemica* F.Muell., a bamboo from northern Australia, by analysis of microsatellites. Three of ten clumps were demonstrably multiclonal, containing a minimum of two, four and five genets, respectively. This raises intriguing questions about the development of clumps and suggests that intergenet competition may at times be overwhelmed by the benefits of coloniality in *B. arnhemica*.

Introduction

In clonal organisms, individuality is a hierarchical and often ambiguous concept with perplexing implications for natural selection (Vuorisalo *et al.* 1997; Watson *et al.* 1997; van Kleunen *et al.* 2002). Nowhere is this more apparent than for clonal plants with a phalanx (caespitose or clumping) growth form. The clumping habit may be a cooperative extension of individuality facilitating resource monopolisation, resource sharing among ramets and long-term persistence (Lovett Doust and Lovett Doust 1982), but crowded ramets may compete for resources (Harper 1985; Hamilton *et al.* 1987; Chesson and Peterson 2002). Bell and Tomlinson (1980) demonstrated arrangements of ramets that appear optimal for resource monopolisation whilst minimising inter-ramet competition, suggesting that the trade-off is indeed critical.

The benefits of clumping to the genetic individual (genet) may be dissipated if shared among genets, and it is often assumed that clumps and genets are synonymous (Lovett Doust and Lovett Doust 1982; Waller and Steingraeber 1985; McLellan *et al.* 1997). Intergenet competition may enhance clumpedness through negative interactions at clump edges (Harper 1985; Sebens and Thorne 1985; Peterson and Jones 1997). Reinartz and Popp (1987) demonstrated that woody clumps of *Xanthoxylum americanum* Mill. averted roots when they encountered conspecifics. Steinger *et al.* (1996) showed that overlap occurred only at the fringes of genets in a caespitose sedge.

Bamboos (Poaceae: Bambuseae) are woody, arborescent grasses. Species with pachymorph rhizomes form clumps (McClure 1966; Makita 1998), of which it has been said that

‘a single seed grows into a tightly packed clone of ramets, effectively excluding other plants from the area’ (Lovett Doust and Lovett Doust 1982). The first part of this proposition remains untested (but see Ratnam *et al.* 1994), and the formation of clumps from more than one seedling has been postulated (Troup 1921).

Here we examine the genetic composition of clumps of the bamboo *Bambusa arnhemica* F.Muell. in the monsoonal tropics of northern Australia by analysis of microsatellites. The species is a feature of flood-prone riparian vegetation in the high-rainfall north-west of the Northern Territory, where it forms often extensive monodominant stands (Franklin and Bowman 2004). That clumps of this species might contain more than one genet was suggested to us by the observation that a minority of flowering clumps contained a spatially structured mix of leafy and flowering culms. As with many bamboos (Janzen 1976), *B. arnhemica* flowers gregariously and dies after flowering, and the timing of flowering is believed to be under strong genetic control (Franklin 2004). The lifespan of *B. arnhemica* is estimated to be 40–50 years. Seedlings emerge at high density and undergo intense thinning (Franklin and Bowman 2003; Franklin 2004). Rhizomes of *B. arnhemica* are of the pachymorph form and mostly less than 30 cm long, so that ramets in vegetatively mature stands are strongly aggregated into discrete clumps (Franklin 2003).

Materials and methods

Study site and samples

We collected leaf samples from ten ramets dispersed around the periphery of each of ten discrete, dense, mature, vegetative-phase

Table 1. Clump characteristics, genotypes and size of alleles found by microsatellite analysis in 10 samples (culms) from each of 10 clumps of the bamboo *Bambusa arnhemica*

Clump no.	Clump area (m ²)	Distance to next clump (m)	Clone identity	No. of clumps	Microsatellite loci and size of alleles (bp)															
					BA02	BA05	BA11	BA17	BA18	BA26	BA29	BA41	BA45							
Mary River																				
1	3.3	4.3	a	10	73	73	209	215	177	177	81	89	147	147	98	100	259	259	91	95
2	4.5	3.5	b	10	67	75	209	215	177	177	81	81	145	149	98	100	259	263	95	95
3	6.3	3.3	c	10	51	69	209	213	177	179	81	89	147	147	100	100	259	261	89	91
4	7.1	12.9	d	6	67	67	209	209	177	177	81	81	147	147	100	100	261	261	89	89
			e	1	67	69	209	215	177	177	81	81	147	147	94	94	261	261	91	95
			f	1	67	67	209	215	177	177	81	81	147	147	100	100	261	261	91	95
			g	1	67	67	209	209	177	177	81	81	147	147	94	94	261	261	89	89
			h	1	67	69	209	209	177	177	81	81	147	147	100	100	261	261	89	89
5	8.2	9.4	i	3	77	77	209	209	177	177	91	91	147	147	96	96	255	267	93	93
			j	3	77	77	209	209	177	177	91	91	147	149	96	96	255	267	93	93
			k	2	77	77	209	209	177	177	91	91	147	147	96	96	255	267	89	89
			l	2	77	77	209	209	177	177	91	91	147	149	96	96	255	267	89	89
Adelaide River																				
6	3.0	4.2	m	10	69	69	211	213	179	179	79	79	147	147	96	100	259	259	97	99
7	4.3	5.2	n	10	69	73	213	213	179	181	79	79	147	147	98	102	255	259	99	103
8	4.5	14.7	o	10	69	73	211	213	179	179	79	79	147	147	96	102	255	255	93	103
9	4.7	2.7	p	10	71	77	211	213	179	179	79	79	147	147	100	100	257	257	91	91
10	4.8	4.2	q	9	77	79	211	211	179	179	79	79	147	147	96	98	255	259	99	105
			r	1	77	81	211	211	179	179	79	79	147	147	96	98	255	259	99	105

B. arnhemica clumps, five in riparian forest on the Mary River (12°55'S, 131°39'E) and five on the Adelaide River (12°56'S, 131°16'E), in March and April 2002. The sampled clumps at both sites were in mono-dominant or co-dominant stands on deep alluvial clay loams and are subject to periods of inundation during most years. The clumps were in stands of uniform age, those on the Adelaide River having since flowered gregariously (2004) and died (D.C. Franklin, pers. obs.). The clumps ranged in area from 3.0 to 8.2 m² (Table 1). Unpublished demographic data from nearby stands suggest that, on the basis of basal area, each clump would contain between 20 and 60 leafy ramets.

Microsatellite analysis

DNA was extracted by the modified CTAB method (Milligan 1992) from 100 mg of leaf sample. Nine loci, corresponding to microsatellite markers BA02, BA05, BA11, BA17, BA18, BA26, BA29, BA41 and BA45, as developed by Kaneko *et al.* (in press), were used for the analysis. Polymerase chain reaction amplifications were performed following the standard protocol of the Qiagen Multiplex PCR Kit (Qiagen, Hilden, Germany) in a final volume of 10 µL, which contained 5 ng of extracted DNA, 5 µL of two by Multiplex PCR Master Mix, and 0.2 µM of each multiplexed primer. Primers were labelled with fluorochromes 6-FAM or VIC (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction amplifications were performed with the GeneAmp PCR System 9600 thermal cycler (Applied Biosystems) by using the following conditions: initial denaturation at 95°C for 15 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing for each of the designed specific primers at 57°C for 1 min 30 s, extension at 72°C for 1 min and final extension at 60°C for 30 min. The size of the PCR products was measured with the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and GENESCAN analysis software (Applied Biosystems).

Results

All nine loci were polymorphic and each demonstrated the occurrence of more than one genotype (Tables 1 and 2). Five loci demonstrated the occurrence of multiple genotypes within

Table 2. Variability among microsatellite loci used for the analysis of clonal composition of *Bambusa arnhemica* clumps

A = the number of alleles at each locus; *G* = the number of genotypes at each locus; and *M* = the number of clumps with multiple genotypes

Locus	Mary River (<i>n</i> = 50)			Adelaide River (<i>n</i> = 50)		
	<i>A</i>	<i>G</i>	<i>M</i>	<i>A</i>	<i>G</i>	<i>M</i>
BA02	6	6	1	6	5	1
BA05	3	3	0	1	1	0
BA11	3	3	1	2	3	0
BA17	2	2	0	2	2	0
BA18	3	3	0	1	1	0
BA26	3	3	1	1	1	0
BA29	4	4	1	4	5	0
BA41	5	5	0	3	4	0
BA45	4	5	2	6	5	0
Mean	3.7	3.8		2.9	3.0	

clumps (Table 2). Two clumps at Mary River and one at Adelaide River were demonstrably multiclinal, the examined culms representing five, four and two genotypes, respectively (Table 1). The multiclinal clumps were the largest of those assessed at each site.

Discussion

This is the first confirmed report of multiclinality within bamboo clumps, a result obtained notwithstanding low levels of polymorphism. Given these levels, there is a considerable risk that not all genets were identified, and our results may thus err on the side of underestimation of the number of genets present. Thus, at least in the case of *B. arnhemica*, clumps and genets are not synonymous as is often assumed (Lovett Doust and Lovett Doust 1982; Waller and Steingraeber 1985; McLellan *et al.* 1997). The present results suggest that clumps of other plant species may contain multiple genets, and also show the need to analyse genet structure within each clump in order to understand the biological and ecological meaning of the clumping habit.

If multiclinality is confined to a minority of clumps, then the clump structure of mono-dominant pachymorph bamboo stands may indeed be the product of spacing behaviour among competing genets. In contrast, intergenet competition in leptomorph (running) bamboos is anticipated to arise as a result of interspersed genets, consistent with their 'guerilla' strategy of growth and exploitation of resources (Lovett Doust and Lovett Doust 1982). Interspersed genets in leptomorph bamboos has been demonstrated (Isagi *et al.* 2004), though not consistently so (Suyama *et al.* 2000).

How multiclinal clumps might arise is an intriguing and unresolved question. One possible mechanism is that clumps may initially have been spatially discrete but subsequently merged with expansion by clonal growth. Even if this is the case, intergenet competition in *B. arnhemica* may be a relatively weak force that can be outweighed by the benefits of colonial living. Strong competition among neighbouring genets should yield adverse growth, as noted by Reinartz and Popp (1987), rather than discrete 'mega-clumps'. Our observation of multiclinal clumps also has potential to explain the occurrence of clumps that have more than one flowering schedule.

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