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Clonal Diversity of *Arundinaria gigantea* (Poaceae; Bambusoideae) in Western North Carolina and its Relationship to Sexual Reproduction: An Assessment Using AFLP Fingerprints

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ABSTRACT *Arundinaria gigantea* (river cane), a native bamboo species, was once abundant in river valleys of western North Carolina. Cane stands are now a rare ecosystem due to land use changes, but restoration efforts are underway. River cane reproduces mainly rhizomatously. Sexual reproduction is often characterized by gregarious flowering, followed by death of the flowering culms and possibly of the attached rhizomes. Suggestions have been made in the literature that clusters of flowering culms are monoclonal, but this has not been tested. In this study, leaves were sampled from sterile and fertile culms along transects from two stands in Jackson and Swain counties, North Carolina. Amplified fragment length polymorphism (AFLP) fragments were generated for these samples using three primer pairs. Most of the resulting AFLP fingerprints generated were not identical; however, using a threshold dissimilarity technique, we estimated the probable number of clones in our samples. The majority of fertile culms within a stand were determined to be monoclonal and distinct from some, but not all, of the sterile culms. Cluster analysis confirmed that most of the fertile culms within each stand were more genetically similar to one another than to the sterile culms, and that the two different stands had distinct sets of genotypes, with no genotype overlap between stands. On the basis of these findings, to maximize clonal diversity and minimize the impact of culm loss after flowering, restoration projects should use propagules collected from multiple stands and from multiple localities within a stand.

INTRODUCTION *Arundinaria gigantea* (Walter) Muhl., commonly known as river cane, is one of three bamboo species native to the southeastern United States where it is broadly distributed throughout (Weakley 2008; see Triplett et al. 2006, for the recently revised taxonomy of *Arundinaria*). In western North Carolina, cane stands, or brakes, grow on sandy floodplains of rivers and streams (Griffith et al. 2007) and are maintained primarily by rhizomatous growth. Large canebrakes are now a rare ecosystem (Noss et al. 1995, Platt and Brantley 1997). Platt and Brantley (1997) describe a complex history of land modification and cane cultivation by native Americans that likely promoted the development of the extensive canebrakes documented in the writings of explorers in the 1800s. In brief, following European contact, native American populations in the southeast declined drastically, and the river cane surrounding Indian floodplain settlements would have invaded their fallow agricultural fields. The Native Americans also practiced 7-to-10-year cyclical burning that helped maintain canebrakes. The decline of these large canebrakes in the southeast appears to have occurred

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quickly after European settlement, due to a combination of overgrazing by domesticated livestock (especially cattle and swine), annual burning, and clearing of floodplain areas for agriculture (Platt and Brantley 1997, and citations therein). In recent times, canebrakes have undoubtedly been cleared for roads and other development. Currently, remnants of canebrakes in western North Carolina are often found in narrow strips bordering rivers and streams and bounded by agricultural fields or roads.

Like other bamboo species, river cane is also known to experience gregarious, monocarpic flowering, possibly on an age-dependent basis (Hughes 1951, Marsh 1977, Platt et al. 2004). Platt et al. (2004) documented 25 flowering events in river cane over 13 growing seasons and concluded that river cane may undergo both gregarious and sporadic flowering. According to Platt et al. (2004), gregarious flowering is typified by a dormant period of a fixed interval followed by a large flowering event that usually results in plant death, whereas sporadic flowering lacks a pattern, and the plant may or may not survive. Janzen (1976), discussing an idealized bamboo semelparous life history, stated that a cohort of bamboo seedlings would grow vegetatively for the same length of time as its parents did and then flower and fruit synchronously, the timing of which is determined by ''an internal physiological calendar rather than an external weather cue'' (p. 354). The actual flowering interval of river cane, if one exists, is unknown. Estimates range from 20–25 yr to 40–50 yr (Marsh 1977, Weakley 2008) based on anecdotal information. Many previous workers simply noted that cane flowered infrequently and sporadically or unpredictably (Marsh 1977). By comparison, the Chinese bamboo, *Phyllostachys pubescens* (Mazel) Ohwi, was observed to have a flowering cycle of 67 yr, with same-aged seedlings flowering synchronously in two different localities where they had been transplanted (Isagi et al. 2004).

In western North Carolina, different river cane stands were observed undergoing sexual reproduction during the spring (April–May) in each of three consecutive growing seasons (2006–08) (Griffith 2008, K. Mathews pers. obs.). Both flowering and fruiting culms were noticed from a distance either by the distinctive purplish color of their spikelets or by the brown leaves and stems of the dying culms compared to the surrounding sterile green culms within the same stand. Fruiting is reported to be much rarer than flowering in river cane (Marsh 1977), but production of grain was observed by at least some culms in each of the flowering stands we encountered, although the viability of the grain was not tested.

Griffith (2008) observed flowering or fruiting patches within river cane stands ranging from a few square meters to hundreds of square meters. Hughes (1951) observed patches of cane flowering (from less than 1% to 6% of a stand) as well as individuals stems flowering and reported that the entire clone (including aerial culms and rhizomes) died in the same year after flowering, a claim substantiated by earlier reports (e.g., West 1934). McClure (1973) observed that both wild and cultivated river cane flowering culms died in the same season that they flowered, along with the rhizomes attached to them. Platt et al. (2004) confirmed that river cane culms usually died within a year of flowering in observations made in Louisiana, Mississippi and South Carolina, but in one case they found culms surviving long after reproduction. Gagnon and Platt (2008) reported that observed flowering culms of river cane in Louisiana always died, sometimes after a one-year lag.

To our knowledge, no studies have been done to characterize the clonal status of flowering river cane. Referring to bamboos in general, McClure (1966; 1973) stated that all the flowering plants in gregarious flowering have a common seed origin. A report of bamboo clones taken from the same plant and carried to different localities flowering simultaneously is given by Kawamura (1927, cited in Marsh 1977). Wells (1932), although probably referring to *Arundinaria tecta* (Walter) Muhl., indicated that gregarious flowering in North Carolina occurred when the cane was all ''from the same parentage'' (p. 61), and Marsh, having studied flowering cane stands in Arkansas, stated that the gregariously flowering culms within a stand ''could represent vegetative spreading from a single individual by means of rhizomes'' (1977, p. 230–231). However, he also observed what he referred to as sporadic flowering, where fertile culms would be scattered throughout an otherwise vegetative stand or where a

culm may bear spikelets at a single node only. Thus it is generally assumed that gregarious flowering within a stand represents the culms of one genet, or clone, because the flowering culms are usually clumped and only patches of cane within stands flower simultaneously, rather than the entire stand (Marsh 1977). However, clumped flowering culms could represent either a single clone or a cohort of same-aged siblings as in the case of *Phyllostachys pubescens* (Nagao and Ishikawa 1998 [in Japanese]), cited in Isagi et al. 2004). In that study, seeds from one flowering event were transplanted to three different locations, and the offspring began to flower 67 yr later at all three sites, but with different behavior: either synchronous flowering and death within one year, or partial flowering followed by successive flowering over three years. Culms from these sites were determined to be of two different genotypes, with flowering times offset by one year, but overlapping and lasting for 2–3 yr each (Isagi et al. 2004).

To investigate questions about the relationship between flowering and clonality in river cane, amplified fragment length polymorphism (AFLP) analysis (Vos et al. 1995) was employed to determine whether culms flowering or fruiting gregariously within each of two river cane stands belonged to the same clone. AFLP analysis has been used for clone identification in other bamboo species (Suyama et al. 2000, Isagi et al. 2004) and in other plant groups (Arens et al. 1998, Escaravage et al. 1998, van der Hulst et al. 2000, Hinkle 2007) because it can potentially reveal a large number of markers with a high degree of reproducibility with no prior genetic information about the group under study. Since flowering in river cane appears to be followed by culm and possibly rhizome death either in the same year or the following 1–2 yr, understanding how synchronized flowering corresponds to the clonal structure of a canebrake is crucial for the management of restored stands. For instance, Isagi et al. (2004) state that in natural forests in Japan dominated by dwarf bamboo species, such as *Sasa kurilensis* (Rupr.) Makino, gregarious flowering of the bamboo and subsequent plant death ''have a remarkable effect on the population dynamics of the seedlings of other plant species, insects, and mammals by changing the light condition on the forest floor and producing large amounts of edible seeds'' (p. 2020). With *Arundinaria gigantea* restoration, we are mainly concerned with perpetuating a restored canebrake for the ecological benefits it may provide, such as stream bank stabilization and runoff uptake, as wildlife habitat (see Platt et al. 2001 for a review), and for sustainable harvest for Cherokee craftmaking (Griffith et al. 2007). Studies have shown that river cane is a highly effective riparian buffer that can improve water quality by trapping sediment and uptaking nutrients (Schoonover and Williard 2003, Blattel et al. 2005, Schoonover et al. 2005, Schoonover et al. 2006). In addition, establishment of new river cane stands or improvement of existing stands may provide pools of genetic diversity needed for successful sexual reproduction. River cane is wind-pollinated and believed to be primarily outcrossing (Judziewicz 1999, Gagnon and Platt 2008), and fragmentation of stands may reduce the potential for gene flow between genetically divergent individuals.

Our goal is to test whether flowering within a stand of river cane is monoclonal or not. If it is, do some culms of one flowering clone remain sterile during a gregarious flowering event? This information should be useful to river cane propagation and restoration efforts, where seeds or rhizomes for propagation may be collected from one or a few existing donor canebrakes, resulting in low clonal diversity of the new stand.

METHODS Leaf tissue was sampled from multiple culms along transects from two stands containing flowering and/or fruiting culms (hereafter referred to as ''fertile culms'' or ''fertile cluster'') in Jackson and Swain counties, North Carolina. The Swain County stand is a large canebrake of approximately 2.89 acres (11,690 m^2) in Bryson City, North Carolina, bordered by the Tuckasegee River and agricultural fields. A cluster of fertile culms, ca. 14.5 m long by 4.5 m wide, within this stand was sampled on May 10, 2006. Since the average river cane clone size was unknown, we wanted to sample intensively along our transects. Two perpendicular transects were made through the center of the fertile cluster along its entire length and width, and leaf samples were collected from the nearest fertile culm every 1.5 m, resulting in a total of 16 fertile culms being sampled. Also, six sterile culms that were encountered

immediately adjacent (i.e., the next culm on any side) to the fertile culms were sampled along these transects, bringing the total number of sampled culms to 22.

The Jackson County stand is a smaller canebrake of approximately 1.46 acres (5,905 m^2) in Cullowhee, North Carolina, bordered by Caney Fork Creek (a branch of the Tuckasegee River) and Caney Fork Road. A fertile cluster, ca. 25 m long by 10 m wide, within this stand was sampled on May 18, 2007. A transect was run through the center of the fertile cluster along its length, and leaves were collected from the nearest fertile culm every 1 m, for 19 m. In addition, two more fertile culms standing approximately 10 m apart from the main fertile cluster were sampled, for a total of 22 fertile culms samples. Also, five sterile culms that were encountered adjacent to the fertile culms along the transect were sampled. On July 18, 2007, we returned to this stand and collected leaves from 10 more sterile culms, which were sampled by walking around the perimeter of the stand as well as diagonally through the center of the stand sampling sterile culms every 5–10 m. This was done to assess the overall diversity of clones within the stand. Thus, a total of 37 culms were sampled at the Jackson County site.

Leaves were collected in the field in plastic vials and transported to the lab on ice, then immediately surface-sterilized with alternating 95% ethanol and bleach washes, following the procedure of Zhang et al. (1997). Leaves were wiped dry and then frozen at −70°C until further processing. Approximately one leaf per sample (ca. 50 mg) was ground to a fine powder in a mortar and pestle using liquid nitrogen. DNA extractions were performed using a modified CTAB/chloroform protocol (Doyle and Doyle 1987), followed by an ethanol/sodium acetate wash.

The AFLP procedure was performed using the AFLP® Ligation and Preselective Amplification Kit for Regular Plant Genomes (Applied Biosystems) according to the manufacturer's protocol. Thus, restriction digests and ligation of adaptors were performed in one reaction using the following reagents: high-concentration grade restriction enzymes MseI (New England Biolabs) and EcoRI (Promega), high-concentration grade T4 DNA ligase (Promega), T4 DNA ligase buffer with ATP (Promega), and double-stranded adaptors supplied with the kit. Reactions were incubated at 37°C for 2–3 hr. This was followed by preselective amplification with primers complementary to the adaptor and restriction site sequence plus one selective base at the 3′ end (MseI + C and EcoRI + A; supplied with the kit), and selective amplification using primers with three selective bases at the 3′ end (MseI + Cxx and EcoRI + Axx) chosen by the authors. The EcoRI +3 selective primers were labeled with fluorescent-dyes. Multiple +3 selective amplification primer pairs were screened for informative variation based on those previously used successfully in *Arundinaria* (J. Triplett pers. comm.) and other bamboos (Suyama et al. 2000). Subsequently, three primer pairs were chosen for analysis of all samples (Table 1). All were labeled with blue fluorescent dye (FAM) because this dye was found to give the most readable AFLP profiles. All polymerase chain reactions (PCR) were performed on a Mastercycler Gradient machine (Eppendorf), either in 0.2 ml reaction tubes or in 96-well plates, following the Plant Mapping Kit PCR parameters. Selective amplification products were electrophoresed on an 3130 Genetic Analyzer (Applied Biosystems) with GeneScan-500(-250) Rox Size Standard (Applied Biosystems) using a 36 cm capillary array. Resulting files were imported into Genemapper vers. 4.0 (Applied Biosystems) software to obtain AFLP fingerprint profiles. AFLP fragments (peaks) of all samples were manually checked and samples were scored for presence or absence of alleles for each primer pair. Amplification of fragments tended to decline after 300 bp in many of our samples, so we did not score fragments greater than this size. A matrix containing presence/absence data for all three primer pairs was constructed for all readable samples. Four samples that did not amplify well were excluded from the analysis. These were all samples from Swain County, including two of the 16 fertile culms and two of the six sterile culms.

Genotypes were analyzed by importing the matrix into the software programs GenoType and GenoDive (Meirmans and Van Tienderen 2004). The program GenoType was used to calculate pairwise Dice similarity measures, transformed to distance measures by the function: $\{1 - [2a/(2a + b + c)]\} \times 100$, where a is the number of bands shared by both individuals, b is the number of bands present

Table 1. AFLP primer combinations and numbers of fragments scored for 55 individuals from two stands of *Arundinaria gigantea*

Primer Pair	Selective +3 Primer Combinations		Number of Fragments	
	EcoRI-	MseI-	Scored	Variable
1	ACA	CAC	191	37
2	ACA	CAA	221	17
3	ACT	CTT	184	17
	All primer combinations		596	71

in the first individual but not the second, and *c* is the number of bands present in the second individual but not the first. A pairwise distance matrix was generated, as well as a histogram of frequencies of pairwise distances.

GenoType was then used to identify the number of clones present after setting a genetic dissimilarity threshold for recognition of genotypes to be considered clonemates or nonclones. AFLP fingerprints have been widely used for clone identification in plants, but a number of studies have shown that clonemates do not always present identical AFLP fingerprints due to laboratory error and/or somatic mutations (Douhovnikoff and Dodd 2003, Meudt and Clarke 2006, Lasso 2008). Researchers have used pairwise AFLP distances of known clones and nonclones for particular plant groups to identify a threshold genetic dissimilarity measure below which samples are considered to represent a single clone (Douhovnikoff and Dodd 2003, *Salix*; Lasso 2008, *Piper*). Alternatively, pairwise genetic distance comparisons among replicate runs or samples have been used to identify a threshold to distinguish putative clones from nonclonal individuals in other studies (Arens et al. 1998, Winfield et al. 1998, both *Populus*). In contrast, the other bamboo AFLP studies (Suyama et al. 2000, Isagi et al. 2004) did not use dissimilarity thresholds or explicit sample replication. Suyama et al. (2000) tested two different sets of control samples and reported that culms from the same clone (confirmed by excavating rhizomes) in each case produced identical AFLP profiles. To set the threshold in this study, we used sample replication to estimate our error rate in scoring AFLP fingerprints. For each of 21 samples, the selective +3 amplification was repeated and the resulting fingerprints were compared between replicates of identical samples. The distance threshold was selected as that which correctly identified replicates of identical samples as belonging to the same genotype.

Simpson's index of diversity corrected for finite sample size (Pielou 1969) was calculated for each stand in GenoDive. This is given by the equation $1 - D = 1 - \{[\Sigma n_i(n_i - 1)]/[N(N - 1)]\}$, where n_i is the number of samples with AFLP profile i and N is the total number of samples. This index ranges from 0 (low diversity) to almost 1. Between-stand genetic diversity was not analyzed because the sample size and sampling strategies were not the same at both sites. In addition, the proportion distinguishable (PD; Ellstrand and Roose 1987) index was calculated. This value is defined as G (number of genotypes)/N (number of samples), or the proportion of genotypes detected divided by sample size. Finally, a neighbor-joining phenogram with 1000 bootstrap replicates was constructed using PAUP* vers. 4.0b10 (Swofford 2003), based on the pairwise distance measure by Nei and Li (1979) for restriction site data.

RESULTS An average of 199 distinct fragments were analyzed for each primer pair, with a total of 596 fragments scored. Among those, 71 (12%) were polymorphic (Table 1). The range of pairwise genetic distances within the Swain County stand was 0.02–0.29, and within the Jackson County stand was 0–0.54. The total range of genetic distances across both stands was 0–0.64. The genetic dissimilarity threshold distance was identified to be 0.09 based on comparing replicate samples. Thus, samples with 91% or greater similarity in AFLP profiles were recognized as clonemates, while samples with 90% or less similarity were considered to be non-clones.

In the genotype analysis, only three groups of genotypes were identical for all three primer sets (including two different pairs of samples and one set of four samples), and

Table 2. Clones 1–12 assigned using a distance threshold of 0.09 (clones recognized at 91% or greater similarity in AFLP profile). Fruiting culms are indicated with an asterisk. Samples from the Jackson County stand are signified by names starting with "J," and samples from the Swain County stand are signified by names starting with "S"

1	2	3	4	5	6	7	8	9	10	11	12
*J0	*J1	*JFA	*JFB	Jb	Je	*S1	*S13	*S9	SS1	SS2	SS3
	*J2				Jf	*S3					
	*J3				Jg	*S4					
	*J4				Jh	*S5					
	*J5					*S6					
	*J6					*S7					
	*J7					*S8					
	*J8					*S10					
	*J9					*S11					
	*J10					*S12					
	*J11					*S14					
	*J12					*S15					
	*J13					SS4					
	*J14										
	*J15										
	*J17										
	*J18										
	*J19										
	Ja										
	Jc										
	Jd1										
	Jd2										
	Ji										
	JS1										
	JS2										
	JS3										
	JS4										
	JS5										
	JS6										

these were all from Jackson County. Based on the threshold value chosen, 12 distinct clones were identified, including six per stand, with no shared genotypes between stands (Table 2). Table 3 shows summary statistics for each stand.

The neighbor-joining tree (Figure 1) distinguished between the two stands, finding separate clusters for each stand, albeit with weak bootstrap (BS) support. The Swain cluster was supported by 57% BS, but the Jackson cluster had less than 50% BS. One outlier from Jackson County (J0) did not group with either stand. Within the Swain cluster branch lengths were relatively short, and only one subgrouping had >50% BS support, indicating close genetic relationships among all the samples from Swain County. The Swain sterile culms were nested with two fertile culms, and they had longer branch lengths than the fertile culm samples.

The Jackson County samples showed greater genetic divergence, as indicated by many long branch lengths in this part of the tree. All the sterile culms but one (JS2) grouped together (BS 72%), and all the fertile culms grouped together (BS < 50%). Within the fertile cluster, branch lengths were similar to those in the Swain cluster. Only one subgrouping of fertile culms had BS support of >50% indicating difficulty by the neighbor-joining algorithm in distinguishing among

Table 3. Summary statistics for the *Arundinaria gigantea* stands sampled in this study

Stand Name	Plot Size (m^2)	Sample Size	Number of Clones Found	Pairwise Genetic Distances	Simpson's Index of Diversity	PD
Swain Co.	65	18	6	0.02–0.29	0.49	0.33
Jackson Co.	5905	37	6	0–0.54	0.38	0.16

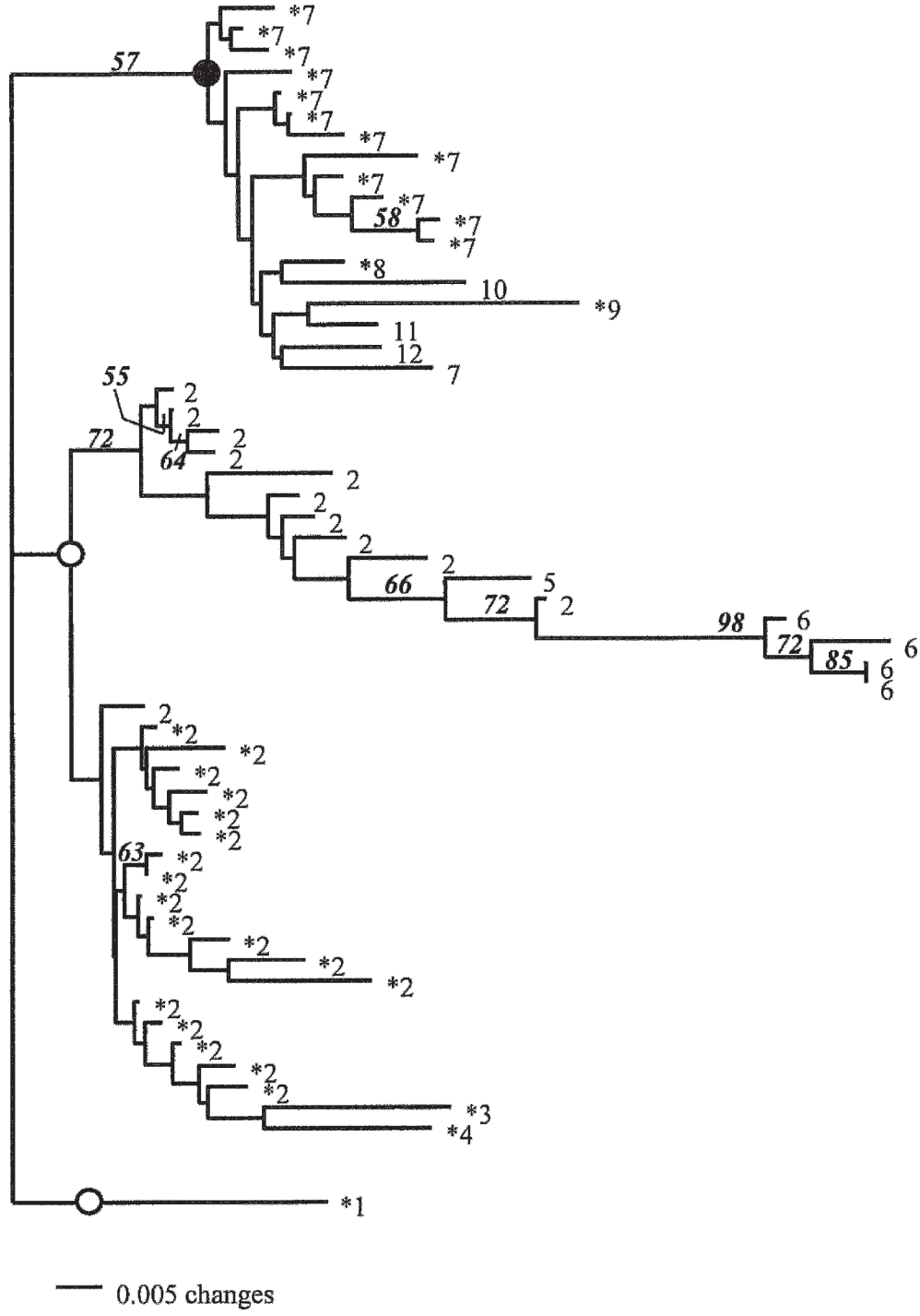

Figure 1. Neighbor-joining phenogram based on pairwise distance measures (Nei-Li distance) of AFLP fingerprints of all *Arundinaria* samples. Samples are indicated by genotype assignment number as given in Table 2. Fertile culms are indicated by an asterisk. The dark circle indicates the Swain County cluster; the open circles indicate the Jackson County cluster and the single Jackson County sample outside this cluster. Bootstrap values greater than 50% are indicated in bold-italic. Branches are drawn to scale, with the bar scale showing changes per character.

those samples. Within the sterile cluster, however, branch lengths were much longer, and the majority of the subgroupings were supported by BS values > 50%, indicating a greater ability of the neighbor-joining algorithm to distinguish among groups of genetically related samples in this part of the tree.

DISCUSSION Our data indicate that within each stand, most of the fertile culms belonged to a single clone. However, 2–3 fertile culms within each stand belonged to different genotypes, according to our dissimilarity threshold value. For the Jackson County stand, this is not surprising, since two of the fruiting culms collected were separated from the main fruiting cluster (JFA and JFB, Table 2); these samples were each identified as having unique genotypes. Furthermore, sterile culms in both stands were identified as belonging to the same clone as fruiting culms, indicating that not all culms within a clone necessarily flower during a gregarious flowering event. These may flower within a year or two of the initial flowering event, as suggested by observations of Platt et al. (2004), and as demonstrated in the study by Isagi et al. (2004), in which two clonal genotypes of *Phyllostachys* flowered over two and three years, respectively. Both our genotype analysis and cluster analysis confirmed that there is genetic differentiation between the Jackson and Swain County *Arundinaria gigantea* stands in that no shared genotypes between stands were found, and the two stands form separate clusters in the phenogram. There are no known studies of between-stand genetic diversity for any bamboo species, and this is an area of research that is needed.

Within the stands, the cluster analysis may provide a more reliable picture of genetic differentiation than the genotype analysis. In the phenogram, clusters correspond more closely to reproductive status than to assigned genotype, particularly within the Jackson cluster where there is an all-sterile subgroup (BS 72%) and an all-fertile-but-one subgroup (BS < 50%). In their study of a planted stand of *Phyllostachys pubescens*, Isagi et al. (2004) were able to distinguish two distinct genotypes out of 41 samples in a 10 m^2 study plot, characterized by the presence of seven and two specific AFLP fragments, which corresponded closely with flowering time. Suyama et al. (2000) detected at least 22 distinct genotypes out of 51 samples in a large (10 hectare), non-flowering stand of *Sasa senanensis* Rehder identified on the basis of 24–83 differing AFLP fragments.

The tree also demonstrates that there is more genetic divergence within the Jackson County stand, but this is certainly due to more extensive sampling at that site. In the Swain cluster, the short branch lengths and low levels of genetic differentiation between the sterile culms and fertile culms indicate that all these samples probably represent a single clone or perhaps several siblings, although genotypes 9 and 10 (samples S9 and SS1) have conspicuously longer branch lengths than the others. There are relatively short branch lengths within the Jackson County fertile culm cluster, with the exception of genotypes 3 and 4 (samples JFA and JFB). There are much greater genetic distances among the sterile culms, indicating the non-clonal nature of many of these samples. This is not surprising since we increased our sampling distance among the sterile culms to 5–10 m (vs. 1 m among the fertile culms), and sampled them from throughout the stand.

Our dissimilarity threshold value of 9% is less conservative than the threshold values estimated in other AFLP studies (2–5%; Meudt and Clarke 2006, Lasso 2008), in that we have a higher error rate than reported in these other studies. Therefore our technique may not be able to discriminate between clones and genetically similar siblings. Comparing the genotypes table (Table 2) to the structure and varying branch lengths shown in the neighbor-joining phenogram (Figure 1), we may be underestimating the actual number of clones sampled in this study. For example, among the Jackson County samples, the samples all designated as genotype 2 fall into two very distinctive clusters, one of fertile and one of sterile culms, although only the sterile cluster received moderate bootstrap support (72%). Therefore we should interpret these data with caution. Future work will encompass greater sampling within the Swain County stand and an assessment of genetic variance partitioning by geographic locality and by reproductive status of culms.

It may be of interest to compare additional genetic diversity indices among different AFLP studies where available, although Douhovnikoff and Dodd (2003) point out that

''comparisons between studies are almost impossible unless sampling schemes can be standardized for stem density and sample spacing'' (p. 1314). Also, Lasso (2007) discovered that calculations of diversity indices are sensitive to changes in the dissimilarity threshold value that may be used in a study. Of three commonly used indices, Simpson's diversity, evenness (how evenly the genotypes are divided over the population) and PD (proportion distinguishable), she found PD to be most sensitive to changes in the threshold and Simpson's index to be the least sensitive (2007).

In this study, the mean Simpson's diversity index for Jackson and Swain County together was 0.44, while in *Sasa senanensis* it was 0.94 (Suyama et al. 2000). The latter was a much larger study site of 10 ha (100,000 m^2), while our two study plots combined were 5,970 m^2, although sample sizes were similar (55 and 51, respectively). Simpson's index was not reported for *Phyllostachys pubescens* (Isagi et al. 2004). In our study, the combined PD was 0.22, in *S. senanensis* it was 0.43, and in *P. pubescens* (10 m^2 plot) it was 0.05. By comparison, Ellstrand and Roose (1987) calculated a mean PD of 0.17 for 21 species of plants, and Douhovnikoff and Dodd (2003) found a PD of 0.22 for *Salix*. Finally, in our study, we detected 12% polymorphism of loci among 55 samples from two stands of *Arundinaria gigantea*. In comparison, Suyama et al. (2000) found a mean of 33.1% polymorphism between culm pairs among 51 samples of *S. senanensis*, while Isagi et al. (2004) found 8.2% polymorphism among 41 samples of *P. pubescens*. All studies used three selective AFLP primer pairs. Standardizing for plot size, the three bamboo studies found 0.02, 0.003, and 8.2% polymorphism per 10 m^2, respectively.

River cane restoration projects should take into account our finding that nearby culms in a stand are likely to be monoclonal or genetically similar to one another. Seeds from a former fruiting event are likely to germinate near the parent plant because they have no dormancy period and viability declines over time (B. Baldwin pers. comm.). Seeds merely fall to the ground beneath the parent plant (Hughes 1951), although they could be moved by rain or floodwater, and seed predation is high (Gagnon and Platt 2008).

Seed viability and the genetic diversity of river cane stands may be influenced by stand fragmentation that results from habitat loss. Woody bamboos are wind pollinated and protandric (a mechanism to facilitate outcrossing) (Judziewicz et al. 1999). Gagnon and Platt (2008) observed that river cane genets flowering individually in isolated patches produced few viable seeds, whereas a wide-scale flowering event in a large stand produced abundant seed with high rates of germination, suggesting self-incompatibility may be a bottleneck in *Arundinaria gigantea* regeneration. If river cane is highly self-incompatible, then successful regeneration from seedlings in restored stands would require the establishment of genetically diverse stands to facilitate outcrossing.

Restoration workers should also be aware that canebrakes from distant localities are likely to contain unique genotypes. To promote genetic diversity and minimize the risk of total stand death following gregarious flowering, canebrakes that are restored by rhizome propagation should take rhizome cuttings from multiple stands, and from multiple locations within stands to prevent sampling one or a few clones. Similarly, seeds that are collected from a single gregarious fruiting event are likely to be similar genetically because the parental fruiting culms are likely to be monoclonal. Thus restoration by seedlings should make use of seeds collected from multiple stands to increase genetic diversity in the restored stand.

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