NIH Grant Proposal

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SPECIFIC AIMS

I propose to begin work on determining the mechanism(s) responsible for flowering induction in bamboos. A five prong approach will be utilized in resolving this aim. My proposal includes three molecular based approaches, one tissue-culture based approach, and one genetics based approach.

The first prong will be the construction of a genomic library of the species *Phyllostachys bambusoides* Siebold and Zuccarini (Japanese Timber Bamboo). (Please see the Significance and Background section for reasons for choosing this species). A combination of cosmids and bacterial artificial chromosomes (BACs) will be used in the genomic library construction with the emphasis placed on cosmids as the primary choice of vector for carrying genomic DNA inserts. This aspect of the project will be carried through to the point of contig assembly based on the sequenced ends of the cloned inserts. The aim of this aspect of the project is to provide a framework by which the genome of *Phyllostachys bambusoides* may be ultimately sequenced.

The second prong will be to make a cDNA subtractive library. This will be done by obtaining RNA-containing tissue from a flowering clone of a bamboo species and hybridizing the mRNAs from it with 1st strand cDNAs of a non-flowering clone of the same species. The bamboo species to be used will have to be determined right at the creation of this cDNA library due to the nature of bamboo flowering incidence. Once the cDNA subtractive library is made, the cDNAs will be partially sequenced to yield expressed sequence tags (ESTs) which can later be compared to databases.

The third prong will be transformation by proxy experiments whereby *Phyllostachys bambusoides* homologues of known flowering-time genes will be expressed in other monocots such as maize or rice.

The fourth prong will be plant tissue-culture based and involve plant growth regulators known as cytokinins. It has already been shown that benzyl-amino-purine (a cytokinin) serves to aid flower initiation of tissue-cultured bamboo plants (Nadgauda *et al*., 1997). I will expand the present body of knowledge to include other cytokinins such as kinetin, for example, in differing concentrations and with differing species of bamboo in order to study the role of plant growth regulators in bamboo flower induction. I will also expand this research to get reliable floral induction in adult-phase explants (Gielis *et al.,*1997b) and not just in seed or seedling (juvenile-phase) derived explants as most of the current research has done (Nadgauda *et al*., 1997a).

The fifth prong will be to import several pounds of bamboo seeds (100,000s of seeds) (Meredith, 2001) of whichever bamboo species has recently flowered (most likely *Dendrocalamus strictus* (Roxburgh) Nees (Male Bamboo) since it usually is in flower somewhere in India and is known to have a flowering cycle of 15-46 years (Janzen, 1976)). The resulting seedlings will be observed for precocious flowering within the first 1-2 years. An attempt will be made to cross those flowering seedlings with each other with the hope of obtaining viable seed of a new strain of bamboo that flowers precociously.

SIGNIFICANCE AND BACKGROUND

General phenology of bamboo flowering

Bamboos have been classified into three categories concerning their flowering behaviour: 1. Annual flowering species, 2. Gregarious (masting) and periodic flowering species, and 3. Irregularly flowering species (Nadgauda *et al*., 1997a). The class of bamboo I am most interested in are those bamboos which fall into category 2. For unknown reasons bamboos in category 2 usually die after flowering. In India, episodes of bamboo masting have resulted in rodent population explosions (Janzen, 1976). However, rodents turn to farmers' crops as soon as the bamboo seed is depleted and the bamboos die. This has resulted in famines for local populations of people (Janzen, 1976) not to mention the effects of rodent-carried diseases such as typhus and bubonic plague (Soderstrom and Calderon, 1976). While understanding the molecular basis of flowering in bamboos may ultimately help Third World people I believe that this information will have a much wider appeal, namely, I think it will help scientists unlock the secrets of our own mortality. Any living clock that counts time in years such as that in *Phyllostachys bambusoides* can potentially serve as a model for any clocklike mechanisms in *Homo sapiens*.

The information gained from sequencing a bamboo genome will help to solve one of the most intriguing phenomena of plant biology, namely, the decades long cycle of growth, then flowering, and subsequent death of bamboo. Scientists have wondered for years the mechanism(s) governing the masting (all plants flower at once around the world) and semelparity (flower, set seed, and die) of bamboo. The semelparous (also known as holocarpic and monocarpic) habit is found distributed in New World bamboos as well as Old World bamboos (Janzen, 1976, Seifriz, 1920) but is not common among the herbaceous bamboos (Judziewicz *et al.,*1999). It occurs in clumping species as well as running (refers to rhizome growth manner) species (Janzen, 1976). Thus the incidence of category 3 flowering (sporadic) occurs infrequently in genera that normally exhibit category 2 flowering. Category 1 flowering is observed normally in many herbaceous bamboos (not specifically mentioned- only inferred from the statement that they are perennial herbs) (Judziewicz *et al*., 1999) and rarely in the woody bamboos, for example, *Bambusa lineata* Munro, *Yushania wightiana* (Nees) R. B. Majumdar (as *Arundinaria wightiana* Nees (McClure, 1966)) and *Ochlandra stridula* Moon ex Thwaites (Gamble, 1896). One herbaceous genus of bamboo, *Eremitis* Doell, even flowers underground in addition to aboveground flowering (Judziewicz *et al*., 1999).

The herbaceous genera *Pariana* Aublet and *Olyra* Linnaeus produce synflorescences thought to attract insect pollinators (Judziewicz *et al*., 1999) whereas most bamboos are wind pollinated (Janzen, 1976). Many bamboo species have barriers to self fertilization but *Bambusa bambos* (L.) A. Voss (as *Bambusa arundinacea* (Retzius) Willdenow)) is not self-incompatible (Nadgauda *et al.,* 1997b). Hayman (1992) discusses a pollination biology scheme possibly unique to the grasses and perhaps functioning in bamboos as well called the S-Z incompatibility system. In it there are two loci, S and Z, each having as many as 40 incompatibility alleles (Hayman, 1992). Essentially the S-Z incompatibility system allows autopolyploids to remain self-incompatible whereas the single locus gametophytic system of dicotyledons does not (Hayman, 1992). However, no S-specific nor Z-specific glycoproteins have been found (Hayman, 1992).

Phyllostachys bambusoides

Phyllostachys bambusoides is the type species for the *Phyllostachys* section of *Phyllostachys* (Renvoize and Hodkinson, 1997). Records and folklore of its flowering cycle have it flowering as long ago as AD 800 (Meredith, 2001). It flowered again in 919 and 1114, sometime between 1716 and 1735, again in 1844-1847, and finally in the 1960s and 1970s (Janzen, 1976). Thus *Ph. bambusoides* has a flowering cycle of about 120 years (Janzen, 1976). It is because of this well established flowering cycle that I have chosen *Ph*. *bambusoides* as a model bamboo to study the phenomenon of flowering induction. It is native to China and was introduced to Japan in the

past (Ohrnberger, 1999). It grows up to 24 m (79 ft) tall (Ohrnberger, 1999) and has been clocked at growing 47.6" in a 24 hr period (Austin and Ueda, 1977). Gielis *et al.* (1997c) report that *Ph. bambusoides* f. *tanake* Makino ex Tsuboi has a DNA content of 4.18 pg per 2C based on flow cytometry. Compare this to *Arabidopsis thaliana* at 0.14 pg and *Zea mays* L. at 11.0 pg (internet #1). *Phyllostachys bambusoides* may be considered a C3 photosynthesis plant since the entire subfamily *Bambusoideae* is classified as having C3 photosynthesis (Hattersley and Watson, 1992). The young shoots of *Ph. bambusoides* are bitter but edible after parboiling several times with water changes (Farrelly, 1984).

Food Aspects of Bamboo

According to Recht and Wetterwald (1992) all bamboos produce edible shoots but Farrelly (1984) mentions that there is a special secretion of a special species of bamboo that has been used as a poison to kill the condemned in China via imbibing of the poison. In further caution, Mollison (1993) advises to get advice as to edible species since many bamboos have a poisonous hydrocyanic acid content. *Guadua angustifolia* Kunth is reported to taste bitter even after cooking (Judziewicz *et al.,* 1999). Also, shoots of *Bambusa multiplex* (Loureiro) Raes ex Shultes & J. H. Schultes are bitter and are rarely eaten (Farrelly, 1984). The edible portion often is the nascent shoot usually harvested while still internally blanched white owing to its subterranean origin. The shoot is also pickled in India and in Japan using lactic acid producing bacteria; thus it is eaten as a fermented product (Mollison, 1993). In the case of *Chusquea aff. culeou* the rhizomes are preferred to the new shoots for eating (Meredith, 2001). The seeds are also used for food when available either directly as a grain (Janzen, 1976) or in the fermentation of an alcoholic drink, *i.e*. bamboo beer, using the rice-like seeds of *Himalayacalamus hookerianus* (Munro) Stapleton (as *Arundinaria hookeriana*) in Sikkim, India (Farrelly, 1984). Bamboo seeds (species not identified) are slightly more nutritious than rice or wheat (Janzen, 1976). Bamboo seeds are not known to contain any toxins (Janzen, 1976). Some bamboos, for example, *Guadua sarcocarpa* Londono & Peterson and *Olmeca spp*. Soderstrom produce fleshly fruits as seeds instead of dry caryopses (like a wheat grain) and the fruits are eaten raw or cooked (Judziewicz *et al*., 1999). Drinkable water is obtained from the internodes of *Guadua* species in Amazonia (Judziewicz *et al*., 1999). Wine bamboo (*Oxytenanthera braunii* Pilger ap. Engler) is used in the production of bamboo wine in Tanzania by cutting a newly extending culm (shoot) and collecting the sap that drips from the cut surface (somewhat similar to collecting maple syrup) and later fermenting it (Meredith, 2001).

Evolutionary Theory

Bamboo is thought to have originated in the Oligocene or Miocene epoch (about 30-40 million years ago) (Meredith, 2001) but the earliest grasses are thought to have co-existed with the dinosaurs in the late Cretaceous period 65 million years ago (Judziewicz *et al.,* 1999). As an aside, there is also a theory that the hominid *Gigantopithecus* ate bamboo as part of his diet much like the panda bear (*Ailuropoda melanoleuca*) does today based on the presence of needle-like phytoliths on fossilized teeth specimens (internet #2). Bamboos are thought to have evolved from an ancestral grass closely resembling *Streptochaeta* Schrad. Ex Nees (Clark, 1997). Whereas most grasses are thought to have evolved in relatively open habitats, bamboos are thought to have evolved in forests where they are very well adapted (Judziewicz *et al.,* 1999). Bamboos are woody (and herbaceous) members of the grass family *Poaceae* (also referred to as *Graminae)* (Clark, 1997). All morphological data (especially the presence of well-developed arm cells in the chlorenchyma) point to a common ancestor for the true bamboos (Judziewicz *et al.,* 1999). Molecular evidence (*ndhF* chloroplast DNA) also supports the hypothesis that true bamboos share a common ancestor (Judziewicz *et al*., 1999). However, molecular evidence points to a split between the Old World and New World woody bamboos (Judziewicz *et al.,* 1999). Molecular evidence does confirm that true bamboos consist of woody as well as herbaceous bamboos (Meredith, 2001). Selection pressures contributing to the evolution of flowering behaviour are thought to include fire (Keeley and Bond, 1999), predator satiation

(Janzen, 1976; Clark, 1997), parasite avoidance, gap creation, and energy partitioning (Clark, 1997).

Genetic Variation

Restriction fragment length polymorphisms were used first by Friar and Kochert (1994) to classify the section *Phyllostachys* according to molecular data (Renvoize and Hodkinson, 1997) and they found wide agreement with previously morphologically classified species within section *Phyllostachys*. Gielis *et al*. (1997a) used random amplified polymorphic DNA (RAPDs) to accurately assess genetic variation and relationships within the genus *Phyllostachys*. A large number of bands was generated and they recommended digitizing gels to handle all the information and to avoid introducing errors from misread patterns.They also recommended that molecular research be concentrated on one reference collection so that errors can be more easily identified and mislabeled plants more easily traced. Genetic variation was found among the bamboo genera *Bambusa* Schreber, *Dendrocalamus* Nees, *Gigantochloa* Kurz ex Munro, and *Thyrsostachys* Gamble using amplified fragment length polymorphisms (AFLPs) (Loh *et al.,* 2000). Internal transcribed spacers (ITSs) of rDNA were not very useful in resolving the genus *Phyllostachys* according to Renvoize and Hodkinson (1997). Other molecular work includes the identification of prolamin genes in *Phyllostachys aurea* Carriere ex A. & C. Riviere (Golden bamboo) (Hilu and Sharova, 1998). Prolamins are the dominant class of seed storage proteins in grasses (Hilu and Sharova, 1998). Their study pointed to widely diverging amino acid sequences between the *Oryza* (rice) and *Phyllostachys* genes. It still has not been determined whether to consider rice an herbaceous bamboo (Veldkamp, 1997).

Seedling populations are known to be highly heterozygous (Nadgauda *et al.,* 1997a). But most, if not all, reports deal with observable phenotypes such as seedling color and seedling size or growth rate (Nadgauda *et al.,* 1997a). Kumar *et al*. (1993) reported that of 400 *Dendrocalamus strictus* seedlings sown only one came up albino and survived until the 4th leaf stage. I have sown seeds of *Dendrocalamus strictus* and have observed variegated seedlings and not just all green or white seedlings. I have also observed a seedling derived red striped culm variant of *Dendrocalamus membranaceous* Munro which normally exhibits a green colored culm. No molecular basis of determining population variation has been done to my knowledge perhaps due to the lack of large populations of bamboo in countries that have the means to do these studies or the lack of molecular labs in countries where natural populations of bamboo are in abundance.

RESEARCH DESIGN AND METHODS

Genomic library construction

Phyllostachys bambusoides is the species of bamboo that is to ultimately have its genome sequenced according to this proposal. In order to mimimize any confusion involving cloned genomic DNA sequences it is important to use as a donor plant for the genomic DNA only a single clone. If multiple sources of plant DNA are used via known cohorts, for example, this could result in unneeded ambiguities due to potential polymorphisms in the DNA sequence. This has the disadvantage of potentially missing some genes, of course, since only one genotype will be used.

The principle of making a genomic DNA library is simple. First, the DNA will have to be extracted (from the leaves), then the DNA will be cut by a restriction enzyme into pieces small enough to be cloned into the vector of choice, *i. e.* cosmids and BACs. The collection of cosmids (individually “housed” in phage and the collection of BACs individually “housed” in a suitable strain of bacteria) are the genomic library. After the libraries have been constructed the component phage or bacteria may be screened using a molecular probe. Such a probe can come from the sequenced ends of the inserted plant genomic DNA or may simply come from a sequence designed to check

for a homologous gene. In this case, probes from the sequenced ends of inserted plant genomic DNA will be generated so as to build contigs based on mimimal overlap. The production of contigs will schematically show the constitution of individual chromosomes based on clones of phage or bacteria retained in the library. Later, complete genomic sequencing may be done to determine the entirety of sequence between the two sequenced ends of the plant genomic DNA insert.

It is best to begin a genomic sequencing effort with a genetic map of the chromosomes as well as with a calculation of how many clones in your library will be needed to have a 95% chance of covering the entire genome (Blumberg, 2001). The base library should have a complexity five times the estimated genome size to have a 95% chance of identifying a clone (Blumberg, 2001). While the method of chromosome mapping was done with *Arabidopsis thaliana* (The Arabidopsis Genome Initiative 2000) and currently being utilized with *Oryza sativa* (Adam, 2000) it will not work with sequencing the genome of *Phyllostachys bambusoides* since a mapping population is not available to construct such a map in the first place. Therefore in the absence of a map it is best to think of the sequencing effort in terms of just "raw" DNA sequence. However, the initial aim of this prong of the project to determine the mechanism for floral induction in bamboo is make a comprehensive genomic library and order this library as much as possible into contigs.

The bamboo DNA will be extracted using already proven methods (Gielis *et al.,* 1997a), *i. e.* CTAB (Loh *et al*., 2000). Then a partial digestion will be performed with a four-base cutter restriction enzyme. This will be followed by a ligation of cut ends to vector arms. Finally, a packaging extract will complete the transfer of the ligated vector DNA and plant genomic DNA into the phage particles. Stratagene (1999) sells cosmid vectors pWE15 and SuperCos1 each of which will hold genomic inserts ranging from 30 to 42 Kbp. Both contain T3 and T7 sequences just outside the insert region so as to facilitate easy sequencing of insert ends. Futhermore Stratagene (1999) sells GigapackIII packaging extracts for genomic lambda and cosmid library construction.

In the case of using BACs, inserts as large as 300 Kbp may be made (Blumberg, 2001). The construction of a BAC library will require the use of pulse field gel electrophoresis equipment and a colony picker (automated laboratory equipment) (Blumberg, 2001). BAC libraries were made in the sequencing effort of *Arabidopsis thaliana* (The Arabidopsis Genome Initiative 2000). This proposal seeks to use both cosmids and BACs in the construction of a genomic library for *Phyllostachys bambusoides* but if insufficient funding is granted then only the cosmid library will be made.

Progress made during the 1st year will include the production of the libraries. The second year will be needed to finish library construction and the third year will involve the assembly of the contigs.

cDNA subtractive library contruction

The second prong to figuring out the molecular mechanism is the construction of a cDNA subtractive library. This will be accomplished by finding a flowering clone of a given species as well as a non-flowering clone of the same species (but possibly different cultivar). For example, *Phyllostachys aurea* 'Albovariegata' (Variegated Golden Bamboo) recently (2000) was flowering in my bamboo reference collection of living plants. However, my clone of *Phyllostachys aurea* (Golden Bamboo) was not in flower. In this case I would have extracted RNA from the synflorescences of *Ph. aurea* 'Albovariegata' (flowering) and extracted RNA from the leaves and branch tips of *Ph. aurea* (non-flowering). Then I would have made 1st strand cDNAs from the mRNAs of the flowering clone. These cDNAs would then be hybridized to an excess of mRNAs of the non-flowering clone. Any double stranded nucleic acids would then be removed (Blumberg, 2001). The remaining single stranded cDNAs would then be made into a cDNA library. Once that library was made ESTs could be made by sequencing the 5' ends of the cDNAs present in the library. All data would be placed into a computerized databank for

comparison with other EST databanks. I think that for this strategy to work best it will be necessary to identify a bamboo just undergoing the floral initiation process. (Or simply try harvesting mRNAs of the flowering plant at different stages of the flowering process). Of course if a plant were to be found at that stage it could be difficult to find another plant not undergoing the same incipient flowering process if the species in question is a masting and semelparous type. But nevertheless, if a cultivar is chosen such as that mentioned above it may make it easier to get a meaningful subtraction. The Southern California Chapter of the American Bamboo Society maintains an extensive collection of bamboos at Quail Botanical Gardens in Encinitas, CA. It should not prove to be problematic at all to find a species of bamboo in flower known to exhibit both masting and semelparity either a Quail Gardens or through the members of the American Bamboo Society.

At the very least information will be generated that will lead to the identification of bamboo floral genes if not the floral induction clock. In the case of rice, it is already known that MADS box-like genes are present in the genome (Kang *et al*., 1998. Jeon *et al.,* 2000). Here the challenge is to go beyond bamboo MADS box genes and try to determine genes of flowering time such as *CONSTANS* of *Arabidopsis* (Onouchi et al., 2000). Certainly it would be a worthwhile effort to probe the cDNA subtractive library for a bamboo homologue of *CONSTANS*. Overexpression of *CONSTANS* in *Arabidopsis* results in earlier flowering in response to long photoperiods. This is interesting because accurate mast seeding bamboos are unknown from closer than about 5 degrees from the equator (Janzen, 1976). This suggests that bamboos are able to count time accurately based on an observable shift in day length. Janzen (1976) further states that internal calendar is without a doubt a genetic trait. Add to this the phenomenon of the bamboo flowering wave where gregarious flowering starts at one location and proceeds directionally across the geographical range of a species (Judziewicz *et al.,* 1999). This was observed for *Chusquea tomentosa* Widmer & L. G. Clark (a clumping bamboo species) (Judziewicz *et al*., 1999), a *Guadua* species (clumper) documented by satellite imagery in Amazonia (Judziewicz *et al.,* 1999), *Bambusa bambos* (as *Bambusa arundinacea*) (a clumper), *Oxytenanthera abyssinica* (A. Richard) Munro (clumper) (Janzen, 1976), and *Ph. bambusoides* (a running bamboo) groves which began flowering in northern Japan and progressively spread to the south (Soderstrom and Calderon, 1976). These examples support the idea of counting time by day length (unless a bamboo species-specific florigen is being released at the onset of flowering). Other known *Arabidopsis* flowering time genes include *luminidependens*, *fca*, and *fve* to name a few that cause a delay in flowering time in wild type *Arabidopsis* under both long and short days (Onouchi *et al.,* 2000). I am interested in discovering flowering-time genes that accelerate flowering initiation and delay it. cDNA subtractive library construction could be done within one year but getting a large number of ESTs will take much longer.

Transformation by proxy

One problem with identifying and cloning putative flowering-time genes is that bamboo has not yet been made amenable to genetic transformation (numerous regeneration protocols have already been established (Ho and Chang, 1998; see Table 1 in Nadgauda *et al*., 1997a, Villegas, 1990) to name a few) in order to test the cloned gene's function. The situation with genetic transformation looks brighter when one looks at the recent improvements in rice transformation such as biolistic bombardment (Christou, 1997) and *Agrobacterium tumefactions*-mediated transformation using the *vir* genes inducer acetosyringone and actively dividing plant cells to improve T-DNA transfer in rice (see Table 1 in Hiei *et al*., 1997). Even electroporation of individual cells with DNA is a possibility worth exploring (Hiei *et al.,* 1997).

Another way to approach the lack of bamboo transformation protocols is to clone putative flowering-time genes (based on homologues from *Arabidopsis* or rice) from *Ph. bambusoides* and transform maize or rice with these bamboo genes. Either unspliced genes (of DNA origin) could be utilized for transformation or fully spliced genes (of mRNA origin) could be used in this strategy of transformation by proxy. Phenotypes to look for would include bamboo-like synflorescences on maize or rice, a delay in flowering in maize or rice, or no flowering at all in maize or rice (at

least not for 120 years!).
It would take the first year to clone all the desired bamboo homologues of known flowering-time genes. Transformation and regeneration of maize or rice would take the remainder of the 3 year time frame allotted for the grant.

Plant tissue-culture and growth regulators

The fourth prong to understanding floral induction in bamboos will be at the tissue-culture level. There have been very few reports in inducing flowering in bamboos using plant growth regulators (Nadgauda *et al*., 1997a, Gielis *et al*., 1997b). Gielis *et al*., (1997b) observed flowering very infrequently in adult-phase explants of several species including *Phyllostachys sp*., *Bambusa tuldoides* 'Ventricosa' (as *Bambusa ventricosa*), and *Chusquea sp*. Their best developed model system is *B. tuldoides* 'Ventricosa' but they did not give the specific details as to the components of their tissue-culture media.

Clearly, inducing flowering in vitro using adult-phase explants is preferable to juvenile-phase explants because of the abundance of adult tissue and because the phenotypes of the adult plants are established. The explant tissue I would use would be the buds at the internodes of small branches. I propose to base my plant tissue-culture research on use of other cytokinins in addition to benzylaminopurine (BA). These other cytokinins would include kinetin, zeatin, dimethylallyamino purine (2iP), and thidiazuron. I would include the species already induced to flower from juvenile tissue including *Bambusa bambos* (as *Bambusa arundinacea*), *Bambusa vulgaris* Schrader ex Wendland, *Dendrocalamus brandisii* (Munro) Kurz, *Dendrocalamus giganteus* Munro, and *Dendrocalamus strictus* (Nadgauda *et al.,* 1997). Additionally, I would include *Phyllostachys bambusoides* and two species of bamboo grown from seeds of plants flowered within the last eight years and are not expected to bloom again for some time: *Otatea acuminata* (Munro) C. E. Calderon & Soderstrom (Mexican Weeping Bamboo) and *Chusquea coronalis* Soderstrom & C. E. Calderon.

A concentration of 0.5 mg/L of BA (in liquid MS media, etc.) was used to induce flowering in *B. bambos*, *D. brandisii,* and *D. strictus* (Nadgauda *et al.,*1997a). Trial concentrations of each cytokinin would have to be performed with each species to get an idea of the thresholds of amounts of cytokinin that may be applied before the tissue suffers necrosis. It is likely that if these other cytokinins can induce flowering it will be at levels far less than those that are lethal. But it is helpful to establish the boundaries o tolerance when working on such an experiment. At this point of the tissue-culture experiment I would not try any combinations of the cytokinins, only experiment individually. The tissue-culture aspect of this proposal could be done within the span of two years. It was reported that several subcultures were done before spikelets emerged in the case with *B. bambos*, *D. brandisii*, and *D. strictus* (Nadgauda et al., 1997a).

Genetics based approach

The fifth prong to determining the molecular basis of bamboo flower induction would be to raise 100,000 or more seedlings of a species like *D. strictus* and select for mutants that flower in the first two years. Janzen (1976) reports that many thousands of *D. strictus* seeds were planted in March of 1895. By April-June of 1896, 5 seedlings had flowered and died (no mention as to whether viable seed was obtained). If a strain of annually flowering bamboo could be developed from a species that normally exhibits masting and semelparity (such as *D. strictus* since the seed that is needed to start with is usually available somewhere in India) it would greatly facilitate studies of the floral induction mechanism as well as a great many other genetic studies since a mapping population would be available for such work.

The experiment would proceed as follows. If a few seedlings flower the first year then crosses could be made with the resulting seed sown almost immediately since bamboo seed doesn't remain viable for very long (Farrelly, 1984) and the progeny in turn can be crossed at the end of the second year assuming that the precocious flowering trait gets passed on to the progeny. At

the end of the second year, the second crosses could be made and the resulting seed sown almost immediately. Thus in the third year one should have a pretty good idea if this approach will work to generate a precociously flowering strain. Once such a strain becomes available it would be feasible to do transformation experiments using bamboo homologues of known *Arabidopsis* and rice-flowering genes. A strong rice or other *Poaceae* member promoter could be utilized to test the expression of these homologues.

Other ideas concerning bamboo flowering

In addition to the above five approachs to understanding the molecular basis of floral induction in mast seeding and semeparous bamboos I offer the following two speculative possibilities of floral induction control. The first is the possibility of a floral induction organelle that controls the onset of flowering. Such an organelle would be likened to the ricinosome (Schmid *et al.,* 2001) or the scintillon (Hastings, 1989), for example. The ricinosome is a plant organelle which has been implicated in programmed cell death. The genes involved in programmed cell death were not discussed in this proposal but are of great importance nonetheless since many times after a bamboo plant flowers, it dies. The future discovery of ricinosomes in bamboo may help to explain the death of bamboo after flowering.

Scintillons are light emitting organelles found in the marine dinoflagellate *Gonyualax polyedra* (Hastings, 1989). Scintillons are under some sort of circadian control since they emit light at night and not in the daytime (Hastings, 1989). So, given that this organelle participates in a time counting mechanism perhaps it is not that unrealistic to think that an organelle exists in bamboo which also counts time but on a longer basis and when that count is reached instead of light being emitted, a signal goes out to the cell nucleus to activate the floral induction genes. It is something to think about.

The second possibility derives from the telomerase theory of ageing. Perhaps there are different types of telomerases being produced in different bamboo species. When chromosomes shorten too much a cascade is triggered sending the plant into reproduction mode. It would be interesting to compare the chromosomes of non-flowering and flowering clones of the same species to see if there is any cytogenetical evidence for flowering induction.

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