Section 3 Moderator's Report

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The opportunity to interact with researchers having a common scientific interest, but with diverse backgrounds, is always rewarding. The 1992 International Chestnut Conference was a remarkable example of such an interaction, with scientific expertise ranging from electron microscopy to forest ecology. It was a pleasure for me to be able to participate. For my comments, I would like to identify some major points that struck me as relevant to the chestnut research community. I also will address some general comments on breeding, as this is an area where my own scientific research may have some overlap. In regard to the presentations on propagation and physiology, I feel that while I learned much about these fields of research over the four days of the conference, my input would be quite naive and probably not too useful.

Richard Jaynes, Albert Ellingboe and Fred Hebard performed a tremendous service in describing the history and current status of chestnut breeding in America. It was particularly striking how the early geneticists working on the problem of blight resistance felt that tree morphology would be a simple genetic trait and that blight resistance would be complex. The subsequent backcrosses to Chinese chestnut become more understandable in this light. The goal of those early workers was correct, however. The loss of the American chestnut was an *ecological* disaster, not simply the loss of a tree species. The primary goal for restoration should still be the development of a blight-resistant tree that is able to occupy the ecosystem niche left by the original American chestnut. Ideally, this tree would be readily interfertile with the surviving trees, in order to retain the location-specific genetic diversity, would rapidly repopulate the forest with a minimum of human intervention, and would be morphologically identical to *Castanea dentata* (Marsh.) Borkh. Toward this end, the experimental plan described by Al Ellingboe is on target, and is firmly based on a large body of genetic experience in crop breeding.

The implementation of the breeding scheme by Fred Hebard is straightforward and well-planned, and is virtually assured of success. However, the desire to retain genetic diversity from the entire range using this method will require repeating the same scheme many times. I believe that it may be worth testing other methods for retaining the genetic diversity at the site where it evolved. Such schemes might involve planting blight-resistant trees which also have genes for male sterility and/or self-incompatibility, in the forest among native flowering *C. dentata* trees. I do not know much about plant male sterility, but it may be possible to force out-crosses to the native trees. Selection in the wild would produce second or third generation individuals with blight resistance that also retain the endogenous allele frequencies.

The retrospective analysis of early experimental plantings by Scott Schlarbaum will be crucial in assessing the success of more recent plantings. The big question that arose concerning these trees is the nature of their survival. I was under the impression that Fi hybrids of *C dentata* x *C. mollissima* Bl. were not field resistant to *Cryphonectria parasitica* (Murr.) Barr, yet a few trees have survived quite well. Knowledge of the true genotypes of these plants would aid in this analysis, as would an assessment of hypovirulence in their cankers. Both talks by James Hill Craddock were instructive on the problems and successes of breeding *Castanea* as a crop. The power of endemic hypovirulence was dramatically shown in his striking photographs of large chestnut orchards. The assessment of cultivars given by Santiago Pereira-Lorenzo gave a marvelous example of the genetic diversity available in the *Castanea* genus. Similar work on chestnut diversity in America and Asia would be very useful over the long run.

Larry Inman and John Elkins were both instructive in describing the difficulties of breeding a long-generation organism, emphasizing theory and practice, respectively. Knowledge of genetics theory will be critical for any future experiments using *Castanea,* whether for continuous improvement of crop varieties or for dealing with the next destructive pathogen that comes along.

John Elkins' field work is especially relevant for testing methods of re-introduction. If possible, future on-site grafting should include control plants to assess the relative success of blight-resistant American clones to known blight-sensitive plants, and to blight-resistant *C. mollissima.*

Scott Merkle and Javier Vieitez described the current state of *in vitro* propagation and manipulation of *Castanea* by somatic embryogenesis. As was recognized by many in the audience, the potential for genetic manipulation for any desired trait makes these technologies very valuable. Unfortunately, the techniques also are very difficult and labor consuming. As a whole, the session showcased many recent advances in *Castanea* genetics. The ability to analyze and manipulate the *Castanea* genome will provide enormous possibilities for crop improvement and disease resistance. In addition, this work will expand our basic science knowledge of how hardwood trees develop, reproduce, and interact with their environment.

A general suggestion for the chestnut research community

The restoration of the American chestnut can be considered a pilot project in the current era of global extinction. Consequently, the techniques and organizations developed for chestnut restoration should be of interest to many groups involved in ecosystem maintenance. Continuity is essential with these long-term projects. In this regard, there is one thing that future scientists will hold current workers accountable for, and for which there is no excuse for failure. That is loss of information. Two types of information are important in the case of the chestnut:

1. *Scientific information.* Chestnut workers can provide a model system for long-term scientific continuity. The American Chestnut Foundation and American Chestnut Cooperators' Foundation should see this as a major role. Old experiments should be reassessed (such as the work described by Mack Morton), and information gleaned from older publications and notebooks. If an experiment has already been done, it is a waste to repeat it because the information was lost. The Cooperators' Foundation, in particular, would be useful in identifying people such as nursery owners, retired foresters and agricultural geneticists, and interested amateurs that can maintain and continue old experiments. Scientific expertise could be provided by a permanent panel of affiliated researchers. The cost would be slight relative to return.

All chestnut and C*yphonectria* projects should be catalogued and updated yearly in the *Journal of The American Chestnut Foundation.* The genetics communities of all modern experimental organisms (for example, *Drosophila melanogaster* Meigen, *Caenorhabditis elegans* (Maupas) Dough., mouse, and maize) have benefited from having small, inexpensive, frequently published news letters to disseminate information within the community. This information is not peer-reviewed, and includes detailed protocols and failed experiments. **I** would like to stress that this network should remain international, include agricultural producers, and encompass work on any members of the *Castanea* genus.

2. *Genetic information.* Of primary importance for the eventual success of restoration of the American chestnut is the preservation of variation in the gene pool. The chestnut is a remarkable case among organisms which have been decimated in the historical past. Most animal species that have been saved from extinction (American bison, whooping crane, cheetah, condor, etc.) have been reduced to very small numbers and have gone through severe genetic bottlenecks. The bottlenecks have greatly reduced the genetic variation in the population.

In the chestnut, the surviving trees are a unique resource in that they retain genetic diversity in the site where those alleles have been selected. Experiments that look at efficient methods of incorporating these genes into breeding populations should be expanded in anticipation of having true breeding blight-resistant American stocks in the near future. Alternatively, methods for long-term storage of pollen, seed, or embryos should be examined.

Although I have emphasized the American chestnut, the above comments also apply to other chestnut species. Every effort should be made to save current agricultural cultivars and wild isolates. The work of Pereira-Lorenzo in Spain and Liu et al. in China are an excellent example of this type of work. The American Chestnut Foundation also may wish to look for funding for a central database of uniform biological material. The database could maintain information on a variety of relevant materials, including fungal strains, agricultural cultivars, wild isolates, hybrids, DNA samples and DNA probes. Ideally, each type of sample would be held at two locations, cross-referenced, and made available for quick access by interested researchers.

Specific comments on future possibilities for chestnut breeding

Modern molecular genetic techniques can revolutionize the rate at which progress is being made in this field. Much work has been recently performed in the mouse, tomato and soybean using interspecific hybrids that takes advantage of the enormous DNA sequence diversity between species. The chestnut has tremendous possibilities because of its fertile interspecies hybrids, many of which are already in hand.

There are two experiments I can suggest that the chestnut research community may want to examine. One is the development of polymerase-chain reaction (PCR) based polymorphic DNA markers which can distinguish species of origin of a plant. These markers will be extremely useful

for identifying the contributions of various species to an individual. This type of assay is essentially a "paternity test" or DNA "fingerprint." Since it is based on PCR, the assay is simple and can use archival materials, fragments of plants sent by mail, embryos, or newly germinated seed. The genetic history of a particular plant can be traced and questions about the contribution of species to the genome can be determined. I estimate a panel of about 40 PCR reactions would be sufficient to analyze the genome. The tests would accurately assess genetic background to, at least, the level of third generation backcross plants. PCR assays are relatively inexpensive, simple and non-radioactive.

The second is the expansion of this set of assays to include approximately 200 simple sequence polymorphic markers between *C. dentata* and *C. mollissima.* When mapped, this number of markers should span the entire genome at approximately 5 cm resolution. Eric Lander's group at the Massachusetts Institute of Technology has developed a highly automated system for doing precisely this experiment (1). This system will undoubtedly be exported from his lab to many medical schools as well as to agricultural companies. I would like to recommend that The American Chestnut Foundation lobby the U.S. Department of Agriculture or a similar government agency to set up a molecular genetics lab to provide this type of service to the agricultural and natural preservation research communities. The start-up price would be too much for any individual research group; however, many people working on a variety of less-studied plants and animals would utilize the service. It would certainly be cost-effective, as the basic technology is identical regardless of the organism studied.

Fred Hebard and Al Ellingboe both alluded to the power of the genomic mapping technique during the meeting. In particular, I would recommend that large numbers of F2 progeny (about 200) be prepared from *known* parental stock and PCR—DNA typed. The results will generate a complete genomic map of *Castanea* (see the discussion in Paterson et al., 1991). An article by Hebard et al. (1991) demonstrates that such numbers of F2 progeny (180 emerged nuts) can be readily produced. For mapping purposes these individuals need only to be grown sufficiently to make DNA, however, they should be grown fully for later phenotypic analysis. F2 progeny are more informative than backcross progeny for constructing such maps (3).

Following completion of the map, a larger total of about 500 F2 progeny should be typed for blight resistance and other phenotypic characters, including quantitative (polygenic) traits such as vegetative growth period, nut yield, resistance to other pathogens, etc. Using an F2 population for these studies is very important for several reasons.

1. One can determine the effect of different gene dosages on genotype because all three possible dosages are represented (i.e., homozygous *C. dentata,* homozygous *C. mollissima,* and heterozygous). This cannot be done in backcross populations which will always lack one of the two homozygous parental dosages. Consequently, an F2 population can map recessive factors from *either* parent, unlike a backcross.

2. Many F2 chestnut plants already exist. Often the true parentage of these individuals must be sorted out; however, DNA typing or fingerprinting should be able to do this rapidly.

3. The same F2 individuals used to generate the original map can also be used for phenotypic scoring of traits.

Since the organisms are F2s, complex traits that involve several recessive genes can be identified, providing that enough individuals are examined. Importantly, the standard calculation used in the backcross experiments (i.e., 3/4, 7/8,15/16) for desired genome per generation can be side-stepped, as was noted by Al Ellingboe. These calculations describe the average gene content of individuals at each generation. In actuality the distribution resembles a bell-curve, with a few individuals having a higher proportion of one or the other genotype. In the paper by Paterson et al. (4), approximately 2% of 350 F2 individuals had genomes which retained greater than 70% of one parental genome.

Complete genomic DNA analysis of the 10% most variant individuals will be able to genetically map the loci involved in the quantitative traits for tree structure, as well as the loci for blight resistance. Genomic analysis will be able to sort these out, and in a single F2 generation (if the number of individuals in that generation is large enough) individuals will be found that have the desired characteristics on the minimum amount of unwanted genome. It should be noted that individuals that do not express the desired phenotype, but retain the desired trait as heterozygotes can be identified by DNA mapping. Breedings can be planned that will use individuals with a higher proportion of desired trait alleles, even if they do not express the traits themselves. Finally, F2 individuals homozygous for resistance can be selectively interbred, and the progeny typed for *C. dentata* genome content. This selected intercross generation (F3) will be homozygous resistant (true-breeding) and may have up to 95% of its genome as *C. dentata.*

The reader may have already done a quick calculation concerning this experiment. DNA typing 200 loci on 500 plants means 100,000 assays (not counting errors and failed typings) to generate a genomic map and identify quantitative trait loci for resistance and tree morphology. At a price of $0.50 to $1 per assay, this is obviously unrealistic with current levels of funding for chestnut. However, the ability to do these experiments 10-fold less expensively is between 5 to 10 years away. Today is an ideal moment to prepare for that eventuality by growing F2 trees, identifying existing F2s, performing standardized phenotype analyses and attracting funding for the project.

It is optimistic, but reasonable, to anticipate having blight-resistant hybrid trees that are genetically greater than 90% *C. dentata,* and phenotypically indistinguishable, within 15 years. It should be noted that all chestnut

breeders would benefit from a genomic map of *C. dentata*. Since interspecific hybrids of all of the agricultural species of *Castanea* are viable and fertile, the genomic maps should be similar (but not exact). The genes that make for good quality and high yield of nuts, as well as resistance to other pathogens also will be identifiable using this basic resource. I would recommend that all of the participants of the meeting work together to support such an effort. Of course, the really nice thing about complete genomic analysis of an organism is that it only has to be done once.

LITERATURE CITED

1. Dietrich, **W.,** Katz, H., Lincoln, S.E., Shin, H.-S., Friedman, 1, Dracopoli, N.C. and Lander, E.S. 1992. A genetic map of the mouse suitable for typing intraspecific crosses. Genetics 131:423-447.
2. Paterson, A.H., Damon, S., Hewitt, J.D., Zamir, D., Rabinowitch, H.D., Lincoln, S.E., Lander, E.S. and Tanksley, S.D. 1991. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations and environments. Genetics 127:181-197.
3. Hebard, F., Rutter, PA., Widrlechner, M. and Inman, L 1991. American Chestnut Foundation 1990 nut harvest. J. Am. Chestnut Foundation 5:60-62.
4. Lander, E.S. and Botstein, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Note: Audio tapes were made during the presentations given at the third session. Unfortunately, the tape containing the talks by L. Inman, S. Merkle, J. Hill Craddock and S. Pereira-Lorenzo did not record property. Anyone interested in copies of the other presentations can contact David Burke at the University of Michigan, Department of Human Genetics, Ann Arbor, MI 48109-0618.