Comparison of Tree Improvement Programs with Crop Breeding Programs

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The title of this talk may appear presumptuous, if nothing else. The hazards of comparing improvement programs with plants as diverse as a loblolly pine and an alfalfa plant at first appear formidable, if not insurmountable. One thinks immediately of the obvious differences in plant size, longevity, generation time, management conditions, etc. From the standpoint of geneticist and breeder, however, these differences are essentially superficial, while in fact the intrinsic similarities between an alfalfa plant and a southern pine are also rather formidable. Both are highly cross-pollinated and rich with native heterogeneity; interspecific compatibilities in each species afford an immense germplasm within reach of the breeder; both crops are bred for maximal productivity with less immediate concern for "type" (as, e.g., in breeding apples); both are long-lived and have unique parasitic and pest problems stemming from this longevity. Perhaps the only real hazard lies in carrying this kind of thinking too far!

Most forest trees are highly outcrossed, some as a consequence of genetic self-incompatibility, and most of them show almost exasperating interspecific compatibilities. Progeny tests reveal extensive genetic variability even from limited and more or less homogenous provenances, a fact which is probably not fully appreciated by silviculturists, forest soils enthusiasts, etc. Rapid and exciting genetic gains have been made in forest breeding during the past decade, especially in the southern United States, capitalizing on this variability through selection and breeding. But a second stage, or a second cycle of improvement, has arrived for the major forest species of the southern United States. With new methods increasingly available to accelerate tree generations, the breeder is or soon will be in his advanced generations of improvement. Few crop breeders have more than a passing acquaintance with this exciting decade of genetic advance in forest tree improvement. Thus it becomes encumbent on the forest breeder to draw his own lessons from the foibles and bobbles of breeders and geneticists working with other, more intensively studied organisms. The fact that I was invited to attempt such comparisons today indicates your confidence in your ability, at least, to profit by the experiences and errors made in this past half-century of intensive breeding advance with other crops.

I've chosen to organize my comments into a series of significant current trends in research in plant breeding and genetics, and attempt to relate them, where possible, to forest breeding problems. It will be obvious that I feel that some of these trends are highly oversold or even irrelevant, while others are long overdue and have not yet been exploited adequately by the breeder.

1. THE TREND AWAY FROM CLASSICAL TYPE

First, there appears to be a strong trend away from what I have chosen to call classical type. Breeders have become increasingly aware of the fact that maximum yields under cultivation are obtained on plants that bear little resemblance to those grown by thefarmer,or to those which nature appears to have favored. An interesting example has been given today in the "silage sycamore," being cut every 2 to 3 years for pulpwood, much as one would a forage crop.

I will long recall a newspaper photograph of famed President Magsaysay of the Philippines, standing proudly in a rice field with an eight-foot rice plant towering over him, a vigor long symbolic of high yields to farmers and agricultural scientists alike. Now, at Rockefeller's International Rice Research Institute in the Philippines they discard, as useless, plants over three feet tall. Maximum yields are obtained from day-length insensitive dwarfs selected for high nitrogen response, and this is for regions in which rice has been grown without fertilization for centuries! This story was born in a geneticist's intrigue with radiation-induced erectoides, dwarfs, reaching its fruition in a revolutionary era of dwarf sorghum breeding. Today it extends to many crops, including corn and vegetable crops; in each case, the trend is toward increased use of fertilizer, increased plant populations, genetic dwarfing of vegetative portions of the crop and

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selection for non-response to day-length variations.

Farmers, plant physiologists, and consumers often have encouraged or compelled breeders to breed within a classical type, partly on the age-old philosophy that nature must have done it that way for a purpose. Of course, an eight foot tall rice plant does compete better in paddies full of weeds, with uncontrolled flooding and no fertilization, but the cultivated crop simply cannot be grown as it was in jungle or neglected pasture.

Most plants are vastly more efficient photosynthetically than they need to be; rice physiologists now tell us that only two healthy leaves can produce enough photosynthate for a plant's entire seed crop. Similarly, plants are often more reproductive than the crop demands, flowering and fruiting abundantly with its consequent drain on vegetative yields. These traits may make a plant compete well in nature, or under conditions of inadequate care and fertilization, but are unnecessary or even detrimental to the cultivated crop.

An example with which I am most familiar occurs in the tropical woody legume, **Leucaena,** which is most commonly found as a pantropical, abundantly flowering, vigorously weedy shrub. Simple selection of non-flowering types from the center of origin of **Leucaena** in Mexico lead to doubling or trebling of forage yields under management. When these non-flowering plants are not harvested, they grow into a handsome tree, not a shrub, attaining over 30 ft. of growth in two years in Hawaii; a potential dual purpose forage-forest plant.

This trend away from classical type received impetus long ago with the recognition of corn breeders that maximal yields were not obtained when selection favored those long, beautiful ears which won prizes at the State Fair. It seems to me this trend away from classical type will see real development as forest tree breeders exploit genes for yield. A tree bred for pulpwood or presswood need bear little resemblance to the same species bred for sawtimber; breeding of non-flowering or sterile hybrids almost certainly would increase vegetative growth in some species; crown shape should be susceptible to dramatic dwarfing without effect on per plant yield, but permit greater plant populations, and you could suggest many other examples.

2. THE TREND AWAY FROM PURE LINES

A second trend which I would like to consider is the trend away from pure lines, and from the homogeneity achieved by prolonged inbreeding or clonal reproduction. This trend is more like a hasty retreat in the cereals, where new mutant races of plant pathogens have forced the breeder to consider new approaches.

Drs. N. Borlaug, N. Jensen and others have long expressed concern about the wisdom of widespread use of pure lines in the self-pollinated cereals, and Syrach Larsen, among others, has expressed a similar concern regarding the use of clones in forest plantings. At the 2nd Forest Genetics Workshop (1964) Dr. Borlaug described his multilineal approach to the cereal breeding problem, allowing the population wide genotypic variability with respect to rust resistance, while maintaining the requisite homogeneity in type. Even corn breeders appear to be retaining greater genetic variability in their varieties and inbreds, especially in the tropics where the pathogenic flora is year-round. Every plant breeding student is familiar with the story of rust race mutations, so I won't belabor it. Its impact on pure line breeding has been greatest in relation to self-pollinated crops and their obligate parasites, particularly where resistance is monogenic. Its relevance to forest tree improvement may be limited.

The major bearing of the trend away from homogeneity on forest breeding might be in the use of clones, as in many hardwoods. An interesting example is obtained in breeding of sugarcane clones at the Hawaii Sugar Planter's Association. Each new clonal variety remains in production for a period of about 15 years, by which time a yield decline, small but significant, has set in. The decline is attributed to the gradual buildup of microflora and phytotoxins, both externally and internally in the roots. Changing clones resolves this problem for the sugarcane breeder, but imposes a continual drain on the resources of the breeder (c. f., Borlaug, 1964).

A clone is clearly more susceptible to the hazards of catastrophic loss todisease and ecologic or climatic disasters, but similar catastrophies are not uncommon at the racial or species levels. Against this problem stands the elegance of clones from the breeder's standpoint, and of their advantage in rate of breeding advance. One cannot but marvel at the intrinsic buffering capacity of single clones in trees such as Cryptomeria in Japan, or commercial varieties of the apple and cherry. The weight of evidence probably favors the ultimate use in forest breeding of synthetics, single crosses or otherwise more heterogenous populations, permitting greater buffering ability to those unforeseen variables of site, climate, and parasites. However, the prospective rate of genetic gain with selected single clones may make their use in early stages of the breeding program most attractive.

3. THE TREND TOWARD REEMPHASIS OF THE SIGNIFICANCE OF SINGLE GENES

Plant breeders often deplore the inadequate training in quantitative genetics gained by today's students, since many genetics courses hardly mention polygenic traits. But one can also deplore a common failure of plant breeding courses to emphasize the dramatic effects which single loci may have on a crop; animal breeders are more cognizant of this than we, with their chondrodystrophic dwarfs, featherless fowls, etc.

Corn geneticists have known for almost 3 decades, about 50 genes affecting the maize endosperm and seed tissues. Biochemists largely ignored them, while breeders regarded them in a class with scute bristles and apricot eyes. Now, one of these genes, opaque-2, has been found to raise the lysine level in the corn seed, giving corn a balanced protein of exceptional nutritional value that can be fed as complete diet to animals. This single gene promises to revolutionize the use of corn throughout the world; since the 1930's it has been just a genetic marker. Today, it appears in the speeches of our Secretary of Agriculture. Many other striking examples of single gene loci, often obtained at considerable expense by the breeder from distantly related species could be cited, such as the dwarfing genes or the determinate flowering genes that permit mechanical harvesting in tomato.

Regrettably, breeders usually study monogenic traits more or less incidentally, because they happen to segregate, or because one of our students wants to see a real 3:1 ratio. Perhaps we are long overdue to seek and study carefully the monogenic variants affecting economically-important traits. In India today, they are screening the world collection of sorghum (over 10,000 varieties) for opaque-like mutants with the high-lysine property. It may have elements of an Edison patiently trying thousands of metals; but then, one cannot question Edison's contribution, either.

4. THE TREND TOWARD USE OF SINGLE-CROSS HYBRIDS

A third trend, if it may be called that, is toward the universality of controlled single-cross hybrids. This is an historic trend, with significant recent additions in the hybrid sorghums, alfalfas, wheats, sugarbeets, cottons, onions, cabbages, and ornamentals. It is a trend often dependent on genetic tools like cytosterility and restorer genes, or sel incompatibility. While it may be immediately applicable to trees like the SI sweetgum and yellow poplar, its application to pines or oaks, for example, may be something else. Perhaps a serious search, however, for cytosterility in gymnosperms would pay off in less time than one might expect.

In corn breeding, the current shift to single-cross hybrids represents a move away from the traditional double-cross hybrids. This has occurred largely since the use of single crosses permits more rapid genetic advance, with only two parents to be altered vs. 4 for the double cross. The corn breeder has been obliged to improve the vigor of his inbreds as seed parents for single crosses, using sib matings with more careful tests of combining ability in lieu of intensive and prolonged self-fertilization. It has become apparent that judicious selection during gradual inbreeding can produce extremely vigorous, high yielding inbreds; the weak, spindly little corn inbreds of our text books are almost a thing of the past.

Single crosses are not yet fully exploited in many crops, such as the forage crops, where there is less value of homogeneity of the cultivated crop. Nonetheless, the trend to single crosses reflects on underlying sense of the generality of the significance of specific combining ability — of the extraordinary interactions of specific single genomes — that is best capitalized upon by the single cross. I think we are on the threshold of some biochemical evidence, from hybrid enzymes, for epistatic and overdominance contributions to heterosis that will enforce upon us the significance of breeding to maximize use of specific combining ability.

5. THE TREND TOWARD USE OF GENETIC DISEASE AND PEST RESISTANCE

Rachel Carson's "Silent Spring" gave renewed impetus to another trend in plant breeding, that of increasing emphasis on genetic resistance to diseases and pests. A major element of this trend deserving our review, is that of monogenic vs. polygenic sources of resistance. Outwardly, the trend might appear to be away from monogenic resistance, and is most evident again in breeding for rust resistance in self-pollinated cereals.

Walker (1964) has emphasized, however, that many forms of dominant, single gene resistance have held up very well without the evolution of new virulent forms; e.g., the resistance of peas and tomatoes to **Fusarium** wilt, of cucumbers to cucumber mosaic and bacterial wilt, and of corn to **Helminthosporium** blight. Classically, these have been single genes which confer a mature plant resistance serving as a check to the pathogen while not inhibiting it completely. The view is encouraged that selection for mature-plant resistance may circumvent the hazard of catastrophe associated with selection for antibiotic immunity. It is always tempting to favor a plant which escapes symptoms entirely; we are perhaps wise to favor seedlings which coexist with a pathogen but fail to succumb to it.

Exciting strides are being made in understanding the biochemistry of disease and insect resistance, and these may be expected to shed light on the breeder's approach. Phenolics and polyphenoloxidases have been associated with resistance to many pathogens (e.g., pinosylvin in blister-rust resistant pines, phloridzin in **Venturia** resistant apples, and other phenolics in resistance of **Beta** to **Cercospora, Daucus to Ceratocystis, Solanum to Phytophthora** and **Triticum to Puccinia).** Isoenzymic approaches, to be described, provide additional potential for understanding pest resistance.

Forest breeders often have the opportunity to exploit genes for resistance that are widely dispersed in the genera with which they work, and this leads me to my next trend.

6. THE TREND TOWARD BROADENING GENE BASE FOR IMPROVEMENT

We have been wisely tutored in the hazards of wide crosses, with emphasis on the mule and his sterility. Plant breeders have demonstrated, however, that the major value of wide crosses within or outside the species is in terms of the genetic bridge that they offer for transfer of desirable gene blocks, not in their use **per se.** We may be impressed with the great difficulty of transferring a given gene, e.g., from the diploid **Triticum aegilops** to highly-selected inbred varieties of hexaploid wheat. At the same time, the problem is different with highly heterogeneous forest trees or forage crops, for example, where a little sterility is of no great concern.

Forest tree breeders can provide exciting examples of this trend to broadening gene bases for improvement, since the interspecies fertilities of forest trees are striking, and the breeder is not so completely dependent on seed or fruit production. Some species crosses in forest trees are of potential value **per se,** as the slash x shortleaf pine crosses. It is fascinating to read of interspecific crosses, for example, that allow a forest breeder to transfer genes **from the Torrey pine, through genetic** bridges **like the Coulter and Jeffrey pines, to the** Ponderosa, or **vice versa.** Evidently the Southern pines are a little more discriminating in their tastes, crossing only with other Southerners, but the scope of usable genetic variability is nonetheless staggering.

I'm well aware that the breeder is soon buried under an avalanche of apparently worthless provenance or species crosses, and it is difficult to convince administrators that all this interspecific junk in the nursery is really worth keeping. Still, I think we should resist efforts to turn aside these interspecific and inter-provenance crossing studies, so long as they do not displace the intra-species programs entirely.

Recognizing that many of our plant species are rapidly disappearing from natural vegetation, the emphasis on germplasm pools has arisen. Clearly, one should preserve seeds or frozen pollen of all forest species crosses, although they might appear of no immediate value, and I hope that some sort of gene bank has been established to this end.

7. THE TREND TO LARGER POPULATIONS FOR SELECTION

Related to the trend toward broadening gene base is a concomitant trend of enlarging the populations on which selection is initially practiced. It is often almost ridiculous how small the initial populations for a selection program are, and little wonder indeed that such trivial progress is achieved. Even with minimal inbreeding, the average breeder would be faced with near-extinction of his materials if he threw out everything he'd prefer to, emphasizing the value of large initial populations in early stages of selection.

Sugarcane breeders in Hawaii, recognizing the great heterogeneity of their material, with its polyploidy and multi-specific base, select about 1000 seedlings a year among over 2,000,000 grown, for an index of selection of about ½%. With clonal increase and evaluation the second year, this selected sample diminishes another 95%. Clonal increase is a powerful tool in selection, but much more so when initial selections represent a truly elite fraction. As an aside here, the sugarcane breeder has a problem with sporadic self-pollination similar to that, for example, in open crosses of pines (e.g., shortleaf x slash), but they choose to ignore natural selfs on the pragmatic stance that if one ever did survive their intense selection, it must be worth evaluation.

Probably no field of plant breeding is currently so preoccupied with problems of individual plant selection as forest tree improvement. It is obvious that clonal and progeny tests are difficult and expensive, impressing the need for highly effective statistical designs. Heritabilities are not yet known for many characters; little assessment has been made of heritabilities with row crops, since they become more or less obvious in large progeny tests. Only with industry's direct participation can forest improvement afford to establish the large populations necessary to permit rapid selection progress; but then industry will be the first to gain by it.

8. THE TREND TOWARD ACCELERATION OF GENERATIONS

Although Hawaii is often described as a "large winter greenhouse," its potentiality as a site for year-round seed production is only beginning to gain recognition (Brewbaker and Hamill, 1967). In our Hawaiian corn breeding, we are disappointed when we fail to get 3 generations per year, an d a Hawaiian forester is disappointed when he fails to obtain 15-20 ft. of growth the first year (in species like the tropical ash, Australian toon, or eucalypts). Other tropical areas have been exploited increasingly by plant breeders in the past decade to permit acceleration of breeding programs. This demand has been most insistent for the breeder of annual crops, who often has well-defined objectives that are 6 to 8 generations away (e.g., conversion to a new recessive allele, like 02).

There are few crops in which breeding objectives and methods are not influenced by accelerations in the seed-to-seed cycle. Methods of grafting, chemical regulation of flowering, and growth in greenhouse have been applied successfully in forest breeding, and perhaps our Hawaiian winter greenhouse will be called upon in the near future. The breeder is well aware of methods to circumvent population-genetic shifts when breeding is conducted out of the region of utilization, and care will be necessary in selecting to avoid too dramatic a shift to early flowering. The potentiality of accelerating genetic gains should encourage all efforts to reduce generation time with forest trees.

9. THE TRENDS IN THE USE OF INDUCED POLYPLOIDS

Breeders are occasionally encouraged by young geneticists to become excited about a hot new technique which promises to revolutionize breeding progress, if only the breeder weren't so stubbornly conservative. Will Rogers used to tell of an eager young county agent who told a farmer, as they leaned over the rail of a pigpen "Do you realize that if you'd boil that corn, the hogs will digest it twice as fast?" The farmer thought about that a minute, then said, "Aw, shucks, that's all right, they ain't in no hurry." Some of the things we learn from modern genetics are about as valuable to the breeder as serving boiled corn to a hog. Nonetheless, we live in an exciting decade in biology (as we are constantly reminded by anyone working on DNA), and the breeder is obliged to ask "what good is all of this?" if only because the molecular biologists aren't likely to ask it themselves.

' Several genetic techniques seem to have been been overrated to the breeder and among these are induced polyploidy and induced mutations. The direct advantages of polyploids, in size, have generally been outweighed by their disadvantages; sterility in the case of seed-producing crops, and reduced growth rate in vegetative crops. In either instance, a major problem is the low rate of genetic advance when breeding at the polyploid level.

The elegant work of people like Sears and Riley has indicated that many polyploids are effectively diploidized by genes controlling intergenomic pairing, and the value to the breeder of these in induced polyploids is encouraged; the rarity of polyploidy in gymnosperms might be taken as a priori evidence that such genes do not occur there.

The early predictions for polyploids were perhaps overstated by some geneticists, yet there are specific instances of their obvious value even in forestry. For gene transfer, induced tetraploids of **Ulmus pumila** are of interest in relation to breeding of the tetraploid American elm, and the aspen triploids are of immediate interest (although the breeder will probably soon provide diploid hybrids to replace the triploids).

A major accession to the use of polyploids is that they should be better buffered against ecologic challenges, etc., but this may be of particular importance only where clones or inbred lines are in use.

10. TRENDS IN THE USE OF INDUCED MUTATIONS

Induced mutations have, it seems to me, been oversold to the breeder. While touted as a source of new genetic variability, or as a means of obtaining a given genetic trait in an otherwise suitable background, induced mutations simply have not paid for the research investment. This is not to question the insight that they have given, and continue to give, into basic phenomena.

Radiation-induced mutants, in particular, regularly carry associated genetic damage that is undesirable and often impossible to circumvent by breeding. Chemical mutagens, like ethylmethanesulfonate, may ameliorate this problem somewhat. The fact is, of course, that most breeders have more genetic variability at their disposal than they know what to do with. If a new trait is desired, induced mutation is often an unlikely and most expensive method of seeking it.

11. TRENDS IN STATISTICAL GENETICS

While the colchicine breeder may be behind us and the mutation breeder somewhat passe, the computer breeder has only begun to rear his head, and some wild predictions of his contributions will doubtless soon be forthcoming. I must only express admiration for the calm predictions of forest biometri clan s.

I once told Dr. H. K. Hayes, my chief at the University of Philippines, that I thought I'd better compute some heritability coefficients on a couple of characters in rice before I set up my selection program. Hayes harumphed as only he could do and said, "Now, Brewbaker, you just get off your .... and spend two months in the rice field, and you won't need to calculate any coefficients at all."

Breeders with the genius and the trained eye of Dr. Hayes never eschew statistics, but never are wed to it either; one often fears that the population geneticist is simply describing in statistical language what these breeders knew all along. Regrettably, the breeder often hasn't said or published what he knows, for he only feels it or senses it intuitively. While we cannot doubt this intuition, we cannot fail to criticize him for not having found a means of communicating it to others. This stems in part from our unrealistic concern, as applied scientists, that we must communicate in the same scientific-sounding language as that of the basic scientist; certainly the social scientist feels under no such compunction, nor should we. Foresters evidently feel that they communicate about as badly as do other agriculturists; somehow the smooth-tongued orators who always pestered the prof in class end up working at the scope or computer, writing monthly articles to Nature, and getting federal money for research of only vague relevance to applied research problems. Nowhere is this sense of the failure of communication so keen as in the gap between statistical geneticist and the field breeder.

Estimates of heritability and of epistatic variance in heterogeneous forest material illustrate, the difficulties of their application as well as one could with any crop. Clearly, heritabilities must be assessed where site variability is minimal, and the immense variation observed between sites is fully predictable. The tree show of clonal material and of selfed or testcross progenies seem much more fruitful in assessing usable genetic variance for selected traits in the long run. I am less impressed now than I would have been a year ago by the need in forest improvement for indices of selection since an initial reaction to forest breeding is that one must work with such small progenies; this is simply not the case. Reviewing the contribution of indices in plant breeding, D. C. Smith (1964) expressed a general disappointment in the application of selective indices for breeding to improve yield. Computer refinements speed calculation, but probably cannot bring sufficient precision for their use except in rare instances. Again this does not militate at all against the contribution of the computer in accelerating the whole process of data retrieval in the breeding program.

The complication of linkage alone has made statistical genetic evaluation of overdominance and epistasis most tenuous even with row crops grown in large populations on extremely uniform sites. This should not disparage such efforts for selected characters in forest trees, but we should not expect a greater impact of these computations in forest breeding than in other crops.

12. TRENDS IN THE APPLICATION OF BIOCHEMICAL AND MOLECULAR GENETICS

The decade just past belongs to the molecular biologist. In its way, it has seemed an era of almost pristine aloofness to the plant breeder's problems, or to problems that cannot be related to the more lucrative ills of **Homo sapiens** or **E. coli.** Biochemical genetics has unraveled much of the mystery of the gene (sensu unicellular organisms), but the fact is that most of the studies of DNA, RNA and so forth have been essentially of no relevance to the problems of the plant breeder. Much has been learned also of biosynthetic pathways, and of the positions that sugars and pigments and other substances occupy on chromatograms; these studies seem also to have shed little light on plant breeding progress.

The improvement of assay apparatus, however, has offered exciting opportunities for cooperative biochemical-breeding efforts, in studies of proteins, enzymes, oils, alkaloids, resin constituents, etc., of more immediate value to the breeder. The application of paramagnetic resonance apparatus in selection for increased oil contents is an example; seeds of many species may be assayed for oil content without affecting their viability, promi sing rapid strides in selection progress. Apart from relating plant chemistry to disease and pest resistance, new techniques may reveal biochemical indicators for graft incompatibility, for epicormic branching tendencies, for heterosis, for sex in dioecious trees, etc. In selecting for heterosis, antigenic heterogeneity has been used by poultry breeders, for example, as indirect evidence of superior combining ability, and we may expect that the level of protein or enzyme polymorphism (i.e., total number of such proteins) might correlate as well with the vigor of a tree's progeny as with the offspring of a rooster or prize bull. We cannot underestimate the important fact that each plant cell is endowed with the tree's entire genotype. The probability must be asserted of stimulating genic (enzymic) expression in seedlings, tissue cultures, etc. normally observed only in mature tissues.

13. A NEW TOOL—GEL ELECTROPHORETIC ANALYSIS OF ISOENZYMES

Having been a little harsh on biochemical genetics in general, it may seem incongruous to conclude with this discussion of the enzymes of plant tissues. However,a new and simple technique has emerged that seems to bring the enzyme into a most significant position in the arsenal of the plant breeder. The majority of my laboratory research is now dedicated to studies using this new technique of gel electrophoresis, and of the enzyme variants called isoenzymes which it reveals in higher plant tissues.

Almost 25 years ago, Beadle and Tatum (1941) drew the remarkably simple but profound conclusion that genes regulated systems in living organisms through their production of enzymes, and that most single genes should be identifiable with single enzymes. To be sure, we can now embellish this statement with much more sophisticated knowledge of DNA, RNA, and proteins. The fact that the

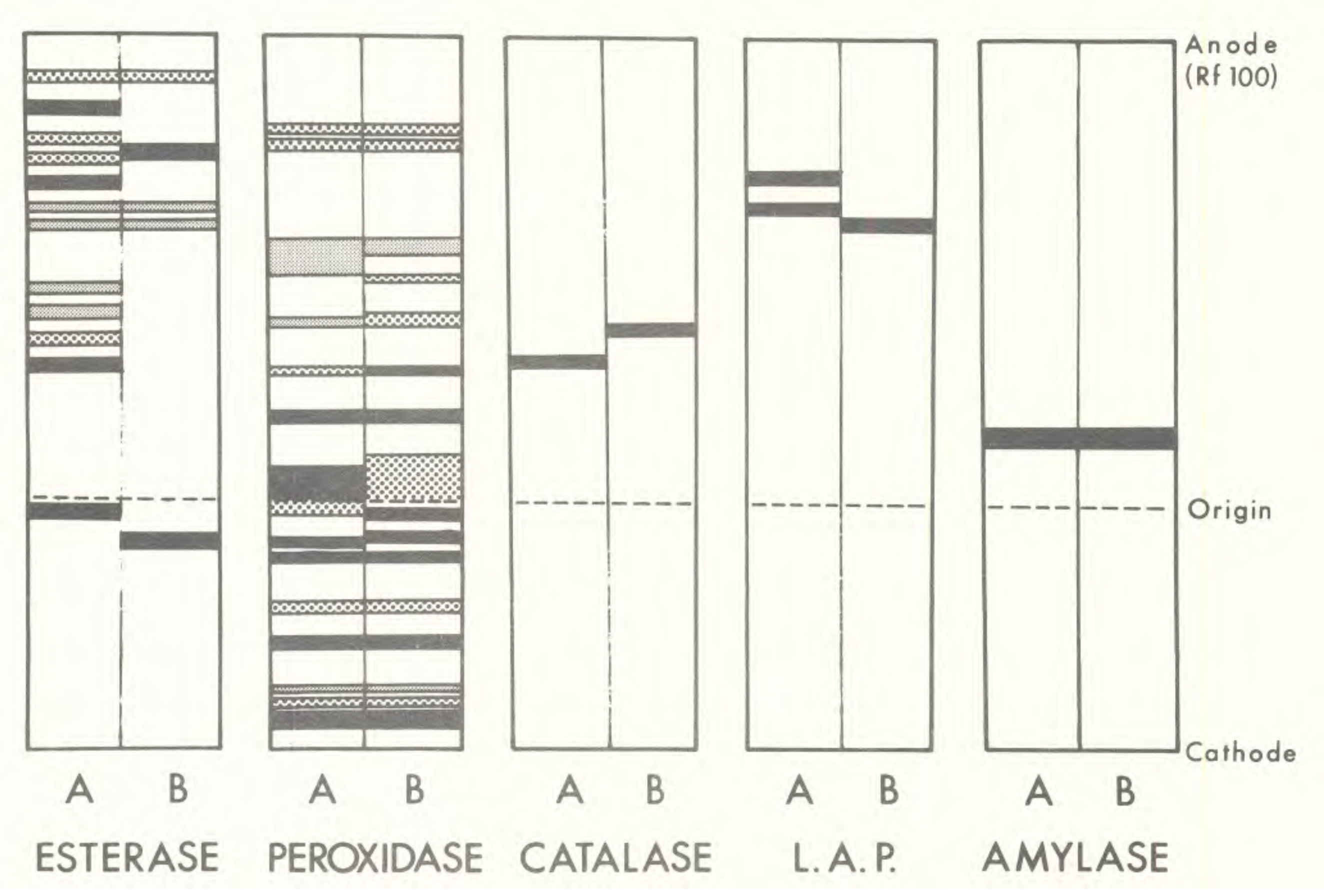

Figure 1 --Some comparative isoenzymic patterns of two corn inbreds (A =Hawaiian sweet inbred AA9; B= Conn. Sweet inbred C42). Starch gel electrophoresis of macerated seedling root.

action of the gene is mediated initially through an enzyme or protein molecule has drawn focus on methods of locating and studying these molecules. Gel electrophoresis provides such a method.

It has long been known that proteins migrate in electric fields, having a positive or negative charge under appropriate pH conditions. In 1955, Dr. 0. Smithies discovered that proteins could be separated most satisfactorily on a gel made of starch which could then be stained for the presence of enzyme bands, a process as inexpensive as it is elegant. We commonly use a starch gel, acrylamide or agar gel about 7" square and¼ " deep. Extraction procedures can be comparatively crude; tissues are often 'macerated in saline, and a ¼" square piece of filter paper soaked with the macerate. About 20 of these papers are inserted into the gel, and 250V (DC) applied for about 4 hours to the gel, a standard refrigerator accommodating about 8 gels. The gels are removed, slit into 2 or 3 wafers, and stained for enzyme systems of interest.

A major limitation of .this system is enforced by the few staining procedures as yet available. Nonetheless, enzymes which may be studied easily are rather important ones, including esterases, peroxidases, amylases, catalases, phosphatases, dehydrogenases, and aminopeptidases (figure 1).

Using the gel technique, we find that even highly purified plant proteins are complexes of many different proteins which can be separated electrophoretically. These separated proteins with the same function are designated isoenzymes by Markert and Moller (1959); they are seen as bands when properly stained on the gel. Purified horseradish peroxidase, for example, contains at least 7 different enzymes, while we find at least 25 different peroxidases from tissues of corn.

Genetic data make it clear that most of these separated isoenzymes are under the control of single gene loci. Some isoenzymes are universally present in tissues of a species, suggesting that they are indispensable; species can occasionally be distinguished by such non-polymorphic loci. Other isoenzymes vary from tissue to tissue or plant to plant, with high activity in some but none in others. Such presence vs. absence patterns are often under the control of "operon" like genes. Many isoenzyme variations prove to be allelic, with alleles at a single locus governing the synthesis of two iso enzymes with slightly different electrophoretic mobility. Generally, hybrids carry the enzymes of bath parent s in selected instances, as in the catalases of corn, the hybrids show bands which we not present in the parents (Beckman, Scandalios and Brewbaker 1964). The ability to identify hybrids from their parents has obvious applications to plant breeding, apart from its intrinsic relevance to the phenomena of heterosis and homeostasis.

The potential applications of isoenzyme techniques in forest tree research appear fascinating, although no one would predict it to be a panacea for problems. Nonetheless, isoenzymes are excellent genetic markers; an inch of root, 1¼ " plug of cambium, or pollen from a single cone provide sufficient material to test for perhaps 60 different isoenzymes (=genes?) of those we have studied (fig. 1). Such genetic markers seem ideal for identification of species hybrids, distinguishing selfs from crosses, or stock from scion wood, etc. Their potential use in evaluating combining ability, graft incompatibility, the tendency to traits such as spiral grain and epicormy, etc., offer intriguing opportunities for 'research. Perhaps electrophoresis %ill not spawn a generation of isoenzyme breeders, but we may nurture the hope that it will help to repair the gap that has widened between biochemical genetics and the problems and progress of the plant breeder.

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