University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Agronomy & Horticulture -- Faculty Publications | Agronomy and Horticulture Department

1969

Germ Plasm Manipulations of the Future

George Sprague
Agricultural Research Service, USDA, Beltsville, Maryland

Follow this and additional works at: http://digitalcommons.unl.edu/agronomyfacpub

 Part of the Plant Sciences Commons

Sprague, George, "Germ Plasm Manipulations of the Future" (1969). *Agronomy & Horticulture -- Faculty Publications*. 192.
http://digitalcommons.unl.edu/agronomyfacpub/192

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Agronomy & Horticulture -- Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Published in *Physiological Aspects of Crop Yield: Proceedings of a symposium sponsored by the University of Nebraska, the American Society of Agronomy, and the Crop Science Society of America, and held at the University of Nebraska, Lincoln, Nebr., January 20-24, 1969*. Edited by Jerry D. Eastin, F. A. Haskins, C. Y. Sullivan, C. H. M. Van Bavel, and Richard C. Dinauer (Madison, Wisconsin: American Society of Agronomy & Crop Science Society of America, 1969). Copyright © 1969 American Society of Agronomy & Crop Science Society of America. Used by permission.

16

Germ Plasm Manipulations of the Future[1]

GEORGE F. SPRAGUE

Agricultural Research Service, USDA
Beltsville, Maryland

The topic, Germ Plasm Manipulations of the Future, could cover the entire field of genetics. This would be a very difficult assignment and, were I willing to attempt it, one that might not best serve the specific purpose of this symposium. I shall take a much more restrictive view and consider only certain phases of genetics which, in my opinion, will become of increasing importance in crop improvement.

In the period since 1900 genetics has achieved close and reciprocally productive relations with several fields of science. A partial listing of these would include genetics and cytology to yield cytogenetics; genetics and statistics to yield quantitative and population genetics; and genetics and biochemistry to yield biochemical genetics. These fields have already contributed greatly to agricultural improvements and their usefulness will undoubtedly increase.

In the past the geneticists interested in crop improvement have been concerned largely with final product evaluation with yield receiving primary attention. Tremendous advances have been made in the last 20 years in experimental design, and they permit an increased degree of precision in the estimation of mean yields. Developments in quantitative genetics have contributed to a better understanding of the genetic basis of variability, and of how such variability can be effectively manipulated in a breeding program. Given a high and a low yielding stock, a study of the F_1 and subsequent progeny permits of certain conclusions as to the genetic difference between the parents. Such an analysis, however, tells us little as to why one parent is low and the other inherently high yielding. Information on the underlying causes of such differences must come from detailed studies combining biochemistry or physiology and genetics. This field has been relatively neglected in crop plants, and it merits greatly increased attention and support. If such an expansion occurs it could well be called biochemical genetics. I would prefer,

[1]Contribution from the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

however, to label it physiological genetics. Obviously the expansion of this field will draw heavily upon biochemistry. It would differ from biochemistry, however, in that biosynthetic pathways and the isolation and characterization of enzymes would not be an end in themselves, but would be extended to explore the effects of individual enzymes or of enzyme systems on all aspects of growth, development and reproductive capacity.

I. THE TRANSFER OF GENETIC INFORMATION

I shall present a very brief and selective review of the tremendous advances made within the broad field of genetics in recent years; selecting only those areas which: (i) illustrate the current state of the art or, (ii) have direct implications to the area I am calling physiological genetics.

The Watson-Crick model for the structure of deoxyribonucleic acid (DNA) has been verified. The genetic code has been solved, at least in its major details. DNA is composed of 4 nucleotides; 2 purines, adenine and guanine, and 2 pyrimidines; thymine and cytosine. The two strands of DNA are complementary and are held together by hydrogen bonding between the purine-pyrimidine pairs; A-T, and G-C. In replication, each DNA strand forms a complementary strand under the control of the enzyme DNA polymerase. Nucleotide sequence is read in triplets (codons). The genetic code resides in the 64 possible arrangements of the 4 nucleotides.

One of the complementary DNA strands serves as a template for the production of an ribonucleic acid (RNA) strand. The DNA specifies the sequence of the nucleotides and controls the production of RNA polymerase. RNA is quite similar to DNA, differing in the sugar backbone of the molecule and in the substitution of uracil for thymine.

Three types of RNA are formed. Messenger RNA is a complementary copy of a segment or the entire DNA template, with the exceptions mentioned above, and therefore carries the genetic information. Soluble or transfer RNA (sRNA) performs the function of supplying amino acids to the developing polypeptide chain in the sequence specified by the nucleotide triplets of the genetic message. sRNA is a small molecule with two active sites; one to attach to the activated amino acid and the other to recognize the codon. Each amino acid has at least one specific sRNA, but some amino acids have more than one. The third RNA type is ribosomal RNA (rRNA). Ribosomes are composed of two subunits, each composed of RNA and protein. The function of rRNA is to bring together the RNA bearing the genetic message and the activated amino acid molecules in the sequence called for by the template codons to produce proteins.

Mutation has been related to one of three events: substitution, deletion, or addition of base pairs within the DNA chain. Genetic changes may also be effected by means of transformation or transduction. (Transformation involves the incorporation of purified DNA, transduction involves the incorporation of DNA by means of virus infection.)

The important developments detailed above have used microorga-

nisms as test material. Two questions logically arise: Are the findings equally applicable to higher organisms, and what is their utility to plant breeding ?

The first question is the easier to answer. Evidence from several types of experiments indicate that the essentials of the genetic code are universal. Protein formation, through the intermediary of the three types of RNA, also appears to be universal.

Genetic control through the use of either transduction of transformation was first demonstrated and has been extensively used in genetic studies involving bacteria. The success achieved has led some to believe that the control of undesirable heritable effects in mankind is just around the corner. If this be true, the same techniques should be available for the improvement of crops on which mankind depends for food, feed, and fiber. For this reason it may be desirable to give further consideration to both transduction and transformation.

Transduction involves the transfer of DNA material (genetic information) to the bacterial genome through the intermediary of phage. The first step in this process involves the incorporation of bacterial DNA into the phage DNA. Under proper conditions this combination of bacterial and phage DNA are incorporated into the genome of a newly infected bacterium. The frequency of such a successful event is very low but because of the large numbers of bacterial cells which can be handled, transduction has proven to be a very useful technique for genetic mapping in both bacteria and phage.

At the moment there is no clear evidence that the same series of events occurs in higher organisms. Most plants are susceptible to one or more viruses. There is, however, only limited evidence to suggest that these viruses can induce genetic changes (Sprague and McKinney, 1966). Where such genetic changes are indicated there is no direct evidence that transduction is the mechanism involved.

Transformation involves the incorporation of DNA, usually after special purification, into the genome of the recipient cell. Such incorporation occurs in low frequency and has been a much less useful tool to bacterial geneticists than transduction.

One would need to be a confirmed optimist to assume that either of these techniques could be useful to the plant breeder in the near future. Several problems would need to be resolved. First, a high degree of control would be essential. Such control would require a greater detail of genetic information involving both host and virus than is currently available or apt to become available in the near future. Without such control, and assuming that the mechanics can be solved, the induced changes would be as random as mutations. Such changes could parallel mutations or duplications, depending upon the length of the DNA molecule involved. Changes of this type can be induced more readily by radiation or chemical mutagens. Secondly, the bacterial chromosome is apparently a naked DNA molecule while the chromosomes of higher organisms have a complex structure. The DNA core is enclosed in a highly organized protein coat. For either transduction or transformation to be both effective and specific this coat must be removed at the appropriate site and time. Problems relating to the effective use of transduction and transformation will eventually be solved, but it appears

unlikely that these methods of transferring genetic material can become useful breeding tools within the next few years.

Biochemical genetics, as developed in bacteria and phage, offers little immediate utility to the plant breeder. This situation arises largely from the general lack of biochemical information leading to a lack of precision in the interpretation of genetic data in the higher organisms. The accumulation of such background information will be a time-consuming operation but one which must eventually be accomplished.

A classical study by Jacob and Monod (1961) may serve to illustrate the power of a combination of biochemistry and genetics in the analysis of an operon. Four genes, occupying adjacent sites on the chromosome, were involved in the study. Each is involved in the utilization of lactose by E. coli. Two of these genes produce enzymes: z produces β galactosidase and y produces a permease which controls the permeability of the cell to lactose. The remaining two genes were designated o and i. The o gene is described as an operator and is adjacent to the z gene. As an operator it controls the action of the structural genes, z and y. In the presence of o^+, normal amounts of the z and y enzymes are produced. In the o^o form, production of both enzymes is inhibited. The common situation in bacteria is for metabolically sequential genes to occupy adjacent sites on the chromosome. The o gene, therefore, may be visualized as the point of origin for a segment of mRNA which specifies the enzymes needed for lactose utilization. The i gene is visualized as blocking RNA transcription of the operator and sequentially related structural genes.

In the absence of a galactoside the organism produces very limited quantities of the enzymes controlled by z and y. In the presence of galactoside, however, enzyme production increases several thousandfold. The i gene regulates this behavior; the wild type allele i^+ is inducible while the recessive allele, i-, is constitutive.

The purpose in presenting this very brief resume of a brilliant research study is to emphasize the detail made possible through the combined genetic and biochemical approach. Had this study been restricted to techniques commonly used in genetic studies with higher plants, most of the details would have remained obscure. Possibly one could have done no more than postulate the presence of a compound locus or a locus with multiple alleles which conditioned the organism's ability to utilize lactose as a carbon source. Thus although we have a great mass of information on the inheritance of traits in higher plants, such information is not directly and immediately useful in an understanding of the underlying biochemical principles and processes.

II. ENZYMES AND HETEROSIS

Heterosis is commonly observed in seedling vigor. Ashby (1930, 1932) has interpreted this early vigor as a reflection of differences in 'initial capital' of embryo tissue in the hybrid seed. This assumption is not supported by data presented by Sprague (1937) or Grossman and Sprague (1948). Therefore the increased dry weight of F_1 hybrid seed-

lings must be related to either greater amounts or greater efficiency of enzyme systems.

Studies on enzymes and heterosis have been under way at the Illinois Station for some years. Much of this work has recently been summarized by Hageman et al. (1967). The general objectives in the studies reported by these workers was to relate certain enzyme systems to heterosis in corn (Zea mays L.). Two groups of enzymes were studied: one group was concerned with the energy transfer system in germinating seeds and young seedlings, and the second with nitrogen metabolism. The first group included the enzymes triosephosphate dehydrogenase (TPD), aldolase (ALD), and glucose-6-phosphate dehydrogenase (G-6-PD). The second enzyme was nitrate reductase (NR) which is involved in nitrogen metabolism.

TPD and G-6-PD are the sites of the first energy conversion; ALD and TPD are involved in the glycolytic pathway relating the stored endosperm starch to the Krebs cycle. Two single-cross hybrids and their inbred parents were utilized in the studies. Hybrid vigor for seedling growth was observed in each cross. In WF9 × M14, hybrid vigor was also observed for TPD and possibly ALD. The hybrids were intermediate for G-6-PD activity. Activity in the Hy2 × Oh7 hybrid was intermediate for all three enzymes. On first consideration these results appear somewhat disappointing. One might have hoped for a greater and more uniform manifestation of heterosis at the enzyme activity level. It must be remembered, however, that the genetic materials used were chosen on the basis of seed availability, rather than because of any prior demonstration of genetic divergence of enzyme activity between the parental lines. Possibly the most that can be concluded is that, for these three enzyme systems, heterozygosity, per se, is not an important factor conditioning the expression of hybrid vigor in the seedling stage.

Considerable information has become available on the response of inbred lines and hybrids to population density. For a variety of reasons tolerance or intolerance of high plant populations was assumed to be related to nitrate reductase (NR) activity. An extensive screening of inbred lines indicated large differences in seasonal means which were reasonably repeatable over seasons. Lines were grouped into three categories: high, medium, and low. High × high, high × low and low × low single-crosses were produced. NR assays were then conducted on these hybrids and their inbred parents. Five of seven low × low crosses gave values exceeding either the mid- or high-parental values. In the high × low category all of the values were intermediate except for one (Hy2 × Oh7) which exceeded the midparental value. In the high × high group none of the NR activity values exceeded the midparental value. In one case, R181 × M14, the value for the hybrid was lower than for either parent. Additional tests reported by Beevers et al. (1964) indicated no qualitative differences among the NR enzymes from several diverse maize sources.

In a later study Warner et al. (1969) a more detailed genetic analysis has been made of one of the low × low crosses exhibiting heterosis for NR activity. The genetic analysis indicates two complementary loci. One locus is assumed to represent a structural and the second a regu-

lator site. Each inbred is dominant at one locus and recessive at the other. In the dark, the enzyme from Oh43 exhibits a very rapid decay rate; three-fold greater than that for B14. In the light, Oh43 synthesizes NR very rapidly while the rate in B14 is much slower. On the basis of the genotypes assumed, heterosis for NR activity would be expected. Similarly, stable F_2 and F_3 lines should be found which possess the activity of the F_1. These expectations were realized.

An increasing efficiency of agricultural production requires a better understanding of nitrogen metabolism. The need for such understanding assumes even greater significance in light of the current world-wide deficiency of protein. We use ever-increasing quantities of nitrogen to maintain or increase yield levels. We know that under many conditions nitrogen fertilization results in an increased protein percentage in the harvested crop. Research findings from the wheat (Triticum aestivum L.) program here at Nebraska indicate that varieties differ in their ability to extract nitrogen from the soil. Genetic differences also exist in the use made of this nitrogen. Some cereals retain higher percentages in the foliage and straw while others transfer proportionally larger amounts of nitrogen to the developing grain to be transformed into protein. In certain stocks genetic control of both quantity and quality of protein has been established. Other aspects of nitrogen metabolism presumably also have a genetic basis but adequate evidence is still largely lacking.

Miflin and Hageman (1963) have developed a technique for isolating chloroplasts from young corn leaves capable of carrying on photophosphorylation. In subsequent studies the same authors (1966) compared three assays: Hill reaction, noncyclic and cyclic photophosphorylation. Comparable results were obtained for each and the cyclic photophosphorylation was used to evaluate a group of inbred lines and their F_1 hybrids. Marked differences were observed in chloroplast activity. The activity of the hybrids tended to be intermediate between the parental values.

The tests by Hageman and his coworkers involving the three enzymes and chloroplast activity indicate a general tendency for the hybrid to be intermediate. These results may be disappointing to those seeking a simple explanation for heterosis. Two points are deserving of emphasis. First, heterosis does not require complete dominance. Cumulative effects of individual loci, each exhibiting values above the midparent, will lead to heterosis. Second, we must remember that no single reaction occurs in isolation in a living cell. Each reaction is modified by both substrate and metabolites. It is not too surprising, therefore, that single enzyme heterosis was not demonstrated. Variability was noted, however, for each of the enzyme systems used and in each case there was evidence that the variation was gene controlled.

Considerable evidence has been reported for the genetic control of isozymes for specific enzymes. Schwartz (1960, 1964, 1965a, 1965b) has reported 7 alleles for the pH 8.6 esterase in maize. In each case the heterozygotes exhibit the parental isozymes and a new hybrid type. This hybrid type does not appear to be formed by an association between the two parental forms. The interpretation of these results is still quite uncertain. In vivo treatment of tissue with sodium borohydride also gives rise to nonparental isozyme forms, paralleling in migration

rate previously identified isozyme forms. Furthermore in vitro treatment with glyceraldehyde converts all isozyme forms to a common type.

Beckman et al. (1964a) have demonstrated the presence of four alleles conditioning isozyme forms of leucine aminopeptidase. In a subsequent paper (1964b) they reported three hybrid catalase isozymes. Scandalios (1965) reported tissue specific isozymes for leucine aminopeptidase, esterase, catalase and peroxidase.

Studies in Drosophila (Ursprung et al., 1968) exhibit a similar pattern but the analysis has been carried somewhat further. Isozyme forms exist for the enzymes aldehyde oxidase and alcohol dehydrogenase. Hybrids exhibit the parental forms and a new hybrid type. A fact of possibly greater significance is that each of these enzymes exhibits a characteristic pattern during development. Alcohol dehydrogenase is low in the egg, increases rapidly during the larval instar stages, falls to a low level during the midpupal stage and rises again during metamorphosis and after hatching. Aldehyde oxidase is high in the egg, falls to a low level during the first larval instar and then increases rather gradually until the adult stage is reached.

The significance of such variations is not clear. The genes responsible are present at all developmental stages. The mechanisms affecting control are the subject of continuing study. The answers may throw light on the whole problem of development.

Stuber and Levings (1969) have demonstrated eight peroxidase isozymes in subapical coleoptile sections of oats (Avena sativa L.). One of these isozymes was repressed by the application of either IAA or 2,4-D. The same growth regulator treatments induced a second isozyme. Genetic variability was indicated for the inducible isozyme. It was suggested that this induction-repression system provides a mechanism for control of the normal growth regulator system.

The results with isozymes are of great interest but many aspects remain unclear. The sometime occurrence of hybrid isozymes is particularly intriguing, because it provides a conceptual basis for overdominance; the hybrid exhibiting a form not present in either parent. However, this attractive possibility thus far receives little support from the published results. This may not be too surprising, since as yet the studies have been concerned primarily with electrophoretic migration rates with little evidence on either qualitative or quantitative characterization of the isozymes involved.

Sarkissian and his coworkers (1966, 1967, 1968) have demonstrated complementation involving mitochondrial preparations derived from inbred lines of maize which, in hybrid combination, produce heterotic hybrids. The activity of the mitochondrial mixture approaches that of mitochondrial preparations from the hybrid in oxygen uptake or oxidadative phosphorylation. The enzymes involved are closely associated with the mitochondrion membrane and have, therefore, not been purified or individually characterized. From the studies thus far reported, complementation appears to be restricted to combinations of lines capable of exhibiting heterosis. As heterosis is commonly presumed to have a quantitative genetic basis, studies of this sort provide some insight into at least one component of the underlying mechanism but do not resolve any of the genetic complexities.

III. MINERAL NUTRITION AND GENETICS

Some genetic information is available on certain aspects of mineral nutrition. Sayre (1955) has demonstrated that inbred lines of maize accumulate mineral elements differentially. This finding has been extended. Gorsline, and his associates at Pennsylvania (1961, 1964a, 1964b), have demonstrated that the differential ear-leaf accumulation of P, K, Mg, Cu, B, Zn, Mn, Al, and Fe was highly heritable. Additive gene effects were of major importance, though varying amounts of non-additivity were also indicated. Ear-leaf and grain accumulations were not correlated, suggesting that different control mechanisms were operative. The possible relation between differential accomulation and efficiency of use remains to be established.

IV. PLANT DESIGN AND BIOLOGICAL EFFICIENCY

Over the years the question has often been raised as to whether our crops are properly designed for maximum efficiency. Initially such queries received little serious consideration. Recent studies, some of which have been reviewed at this Symposium, indicate consideration must be given to both plant design and function.

Several studies have indicated the importance of leaf angle and crop canopy on grain yield and apparent photosynthetic efficiency. I shall cite only a few of the studies in this area which appear to have strong genetic implications. Pendleton et al. (1968) compared isogenic single crosses differing in leaf angle. The hybrid having nearly upright leaves gave a significant increase in yield amounting to about 40%. A simply inherited character, $\underline{lg}_2$, was used to provide the differential for leaf angle. The significant difference in yield is the more impressive as many simply inherited traits tend to condition pleiotropic effects, some of which are deleterious. Somewhat less striking effects were obtained with a normal hybrid through an artificial change in leaf angle. In this case, yield differences were less pronounced, but the plots in which the leaves were in an upright position tended to produce increased yields. Wide differences in leaf angle can be observed in any corn breeding nursery. If this trait has the importance indicated by the studies of Pendleton et al. commercial utilization should not be difficult or long delayed.

Eastin and Sullivan (1969) have demonstrated a significant difference in apparent photosynthetic efficiency by compact and open-headed sorghum types (Sorghum vulgare). These same studies indicated the importance of the flag leaf as an important photosynthetic organ.

V. THE PHYSIOLOGICAL GENETICS APPROACH

The preceding review has dealt briefly with certain aspects of physiology, biochemistry, and genetics. It will have served its purpose it if calls attention to two facts. First, there is a growing body of evi-

dence either establishing or suggesting that many conditions related to biological efficiency are under genetic control. Second, the body of such evidence is quite inadequate to indicate the maximum efficiency which may be attainable. There can be little question but that increased activity in this area would be highly productive.

Physiological genetics, as I am using the term, would be a very broad and somewhat utilitarian discipline. It would include any aspect of physiology involving form or function which has an influence on economic worth or biological efficiency. Similarly it would include any aspect of genetics that was required for the efficient manipulation of the traits of interest. Progress, therefore, will be highly dependent upon the close cooperation between research workers in these two fields.

Differences in inherent yielding ability (dry matter production) could arise from inefficiencies in any one or more of the following broad areas: (i) energy transfer mechanisms; (ii) net assimilation rate; (iii) translocation and utilization of photosynthate; (iv) nutrient uptake and use; (v) plant growth substances; (vi) response under stress conditions; and (vii) efficiency of water use. The investigator choosing any of these areas for detailed study would make extensive subdivision based on both relative importance and feasibility of study. My knowledge of physiology is too limited to permit any meaningful suggestions.

Two factors of great importance deserve stress. First, if the research is to be productive in terms of physiological genetics, it is imperative that a simple and rapid analytical procedure be available for any reaction or trait of interest. Ideally, this analytical procedure would have a high degree of repeatability to minimize sampling and environmental problems. This is a major requirement because the genetic analysis and possible eventual incorporation into breeding stock will require large numbers of determinations. Second, an extensive survey of the germ plasm of the species should be undertaken to identify the range of variability that exists. The assessment of the potential contribution to biological efficiency can be studied more readily when extreme types are involved. Each enzyme of a system may be subject to individual induction or repression so mean or net values may have little value or meaning. Natural selection would have operated on the system as a whole, so that a common end product provides no assurance that the component steps are also equal.

I shall turn now to the more strictly genetic aspects which may be required. Information is required on the influence of the two alternative states (low activity vs. high activity, low leaf angle vs. high leaf angle, etc.), on plant performance; and the more favorable characteristic must eventually be introduced into superior breeding stocks. In some cases these two objectives may be achieved through one transfer operation. Under other circumstances two separate operations may be required and we shall consider the two operations separately.

Appropriate techniques for comparing strains differing in efficiency of a particular enzyme or some other trait of interest will be influenced by the complexity of inheritance, the sources of the contrasting types, and the time requirements of the analytical procedures. It would appear that if the trait involves the primary gene product (an enzyme)

inheritance will tend to be monogenic. The greater the number of steps between the primary product and the expression of the character the greater will be the likelihood for multigenic inheritance or interactions.

If inheritance is simple the most efficient evaluation of differences will be obtained through use of isogenic lines. Several sets of such lines, differing in origin, would be desirable to minimize the background genotype effects. This approach has two possible limitations. First, the term isogenic is relative rather than absolute. Even long-time inbred lines exhibit some degree of genetic variability, presumably arising through mutation, so that either long-continued selfing or backcrossing can only be expected to narrow but not completely eliminate extraneous genetic differences. Second, the development of such contrasting pairs is a time-consuming operation requiring identification of the contrasting types in each segregating generation.

If the trait of interest occurs in adapted lines, a diallel analysis may supply the desired information. This method requires the utilization of a series of "high" and "low" lines to permit the expression of phase of the trait at the F_1 level. This method suffers from the limitation that the variances estimated are influenced by the lines comprising the diallel set. Therefore, interpretations must be made with some degree of caution.

Under conditions where one phase of the contrast of interest is rare, comparisons may be made in the F_2 generation. This approach requires that each F_2 plant be assayed. A simple analysis of variance involving the 'between-' and 'within genotype' contrast provides the significance test of interest. If the F_2 population is large, one may assume, except for linkage, that extraneous genetic differences are averaged within each subpopulation, thus minimizing the background genetic influence.

If the trait is inherited in a quantitative manner alternative procedures must be used. The development of isogenic lines becomes impractical or impossible. The diallel approach remains a possibility but one must be concerned with covariances as well as variances. This is true because the F_1 array will exhibit a range in expression of the trait under study. It would be desirable to include the parental types in the diallel analyses (Griffing, 1956) which would permit the estimation of both reciprocal and parent-progeny relations.

The examination of F_2 populations may also be useful. As the array will represent a continuous distribution rather than two discrete classes a different system of analysis is required. A simple regression of Y on X would appear to be appropriate.

Additional tests and details of suitable experimental designs may have to be developed to fit special needs or circumstances.

After the establishment of the superiority of the variant type the problem of incorporation into useful breeding stocks remains. This task requires the continued cooperation of the physiologist and the geneticist. The complexity of inheritance of the trait under study as well as breeding objectives will influence the choice of transfer methods. Basically the choice lies between incorporation into existing populations of known characteristics (lines, varieties, synthetics or compos-

ites) or the development of new populations with the attendant task of evaluating merit for other traits of agronomic importance. The first alternative will normally be the most economical in terms of total effort required.

In a recent review (Sprague, 1966) of Quantitative Genetics in Plant Breeding, I have used the term population improvement as a general term to include all operations within a system designed to develop an improved type, whether this be a random mating population or an improved line. The procedures reviewed there in some detail encompass the several alternatives that would be most useful in the present context.

If the trait is simply inherited, transfer to a stable line or composite is readily accomplished by backcrossing. The heterozygous individuals must be identified in each generation through either appropriate genetic tests or physiological evaluations. Transfer to a stable inbred line poses the fewest problems. Such a procedure, however, should be viewed as a short-term solution. Relatively few inbred lines or self-pollinating varieties have a long expectancy of commercial usefulness. Furthermore genetic modifiers peculiar to the recurrent parent may limit the expression of the desired trait. Transfer to random-mating populations (natural or induced) will normally provide the most satisfactory long-term solution. Obviously the populations chosen as recipients should represent the best synthetics or composites currently available and preferably those which are undergoing active selection for further improvement.

The two objectives, further improvement and incorporation, are not incompatible. The donor strain can be grown as a separate entry within the recipient population and hand-emasculated or detasseled depending upon the morphology of the crop. The crossed seed would then be used to produce an F_2 generation. The desired individuals within this population could again be crossed to the current generation of the recipient population. Thus the incorporation utilizes, in each back-cross cycle, the most advanced generation of the recipient population. After the required number of generations of backcrossing a new subpopulation can be derived which is homozygous for the trait of interest but which otherwise carries the gene frequency of the recipient population.

If the desired attribute is conditioned by many genes, transfer of the entire complex by backcrossing will be very difficult. Under such conditions some type of recurrent selection or an alternation of backcrossing and recurrent selection may be appropriate. The final choice of procedures can best be made after detailed information is at hand on mode of inheritance.

In this brief review I have not attempted any long-range projections for new genetic techniques which may reach the stage of usability. I have not attempted to give detailed information on many aspects of genetics; present developments of theory far exceed their effective utilization. Rather, I have attempted to indicate that the available genetic knowledge is adequate to provide the necessary support of a productive cooperative effort involving physiology and genetics. A major expansion is needed in this combined area to provide: (i) a better understanding of crop response, and (ii) a more sound basis for further in-

creases in agricultural efficiency. The research required will demand time, funds, and facilities but should achieve a high order of productivity.

LITERATURE CITED

Ashby, E. 1930. Studies in the inheritance of physiological characters. 1. A physiological investigation of the nature of hybrid vigour in maize. Ann. Botany (London) 44:457-467.

Ashby, E. 1932. Studies in the inheritance of physiological characters. 2. Further experiments upon the basis of hybrid vigour and upon the inheritance of efficiency index and respiration rate in maize. Ann. Botany (London) 46:1007-1032.

Beckman, L., J. G. Scandalios, and J. L. Brewbaker. 1964a. Genetics of leucine aminopeptidase isozymes in maize. Genetics 50:899-904.

Beckman, L., J. G. Scandalios, and J. G. Brewbaker 1964b. Catalase hybrid enzymes in maize. Science 146(3648):1174-1175.

Beevers, L., Dounce Flesher, and R. H. Hageman. 1964. Studies on the pyridine nucleotide specificity of nitrate reductase in higher plants and its relationship to sulfhydryl level. Biochem. Biophys. Acta 89:453-464.

Eastin, J. D., and C. Y. Sullivan. 1969. Carbon dioxide exchange in compact and semi-open sorghum inflorescences. Crop Sci. 9:165-166.

Gorsline, G. F., J. L. Ragland, and W. I. Thomas. 1961. Evidence for inheritance of differential accumulation of calcium, magnesium, and potassium by maize. Crop Sci. 1:155-156.

Gorsline, G. W., W. I. Thomas, D. E. Baker, and J. L. Ragland. 1964a. Relationships of strontium-calcium accumulation within corn. Crop Sci. 4:154-156.

Gorsline, G. F., W. I. Thomas, and D. E. Baker. 1964b. Inheritance of P, K, Mg, Cu, B, Zn, Mn, Al, and Fe concentrations by corn leaves and grain. Crop Sci. 4:207-210.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463-493.

Grossmann, A., and G. F. Sprague. 1948. Comparative growth rates in a reciprocal maize cross. 1. The kernel and its component parts. J. Amer. Soc. Agron. 40:88-98.

Hageman, R. H., E. R. Leng, and J. W. Dudley. 1967. A biochemical approach to corn breeding. Advance. in Agron. 19:45-86.

Jacob, F., and J. Monod. 1961. On the regulation of gene activity. Cold Spring Harbor Symposium 26:193-209.

McDaniel, R. G., and I. V. Sarkissian. 1966. Heterosis: Complementation by by mitochondria. Science 152:1640-1642.

McDaniel, G. F., and I. V. Sarkissian. 1968. Mitochondrial heterosis in maize. Genetics 59:465-475.

Miflin, B. J., and R. H. Hageman. 1963. Demonstration of photophosphorylation in maize chloroplasts. Plant Physiol. 38:66-70.

Miflin, B. J., and R. H. Hageman. 1966. Activity of chloroplasts isolated from maize inbreds and their F_1 hybrids. Crop Sci. 6:185-187.

Pendleton, J. W., G. E. Smith, S. R. Winter, and T. J. Johnson. 1968. Field investigations of the relationships of leaf angle in corn (Zea mays L.). Agron. J. 60:422-424.

Sarkissian, I. V., and H. K. Skrivaslava. 1967. Mitochondrial polymorphism in maize. II. Further evidence of correlation of mitochondrial complementation and heterosis. Genetics 57:843-850.

Sayre, J. D. 1955. Mineral nutrition of corn. In G. F. Sprague (ed.) Corn and corn improvement. Agronomy 5:293-314.

Scandalios, J. G. 1964. Tissue specific isozyme variations in maize. J. Hered. 55:281-285.

Schwartz, D. 1960. Genetic studies on mutant enzymes in maize: Synthesis of hybrids enzymes by heterozygotes. Proc. Nat. Acad. Sci. USA 46:1210-1216.
Schwartz, D. 1964. Genetic studies on mutant enzymes in maize. V. In vitro interconversion of allelic isozymes. Proc. Nat. Acad. Sci. USA 52:222-226.
Schwartz, D., Lucy Fuchsman, and K. H. McGrath. 1965a. Allelic isozymes of the pH 7.5 esterase in maize. Genetics 52:1265-1268.
Schwartz, D. 1965b. Genetic studies on mutant enzymes in maize. VI. Elimination of allelic isozyme variation by glyceraldehyde treatment. Genetics 52:1295-1302.
Sprague, G. F. 1937. Hybrid vigor and growth rates in a maize cross and its reciprocal. J. Agr. Res. 53:819-830.
Sprague, G. F. 1966. Quantitative genetics in plant improvement, p. 315-354. In K. J. Frey (ed.) Plant breeding. Iowa State Univ. Press.
Sprague, G. F., and H. H. McKinney. 1966. Aberrant ratio: An anomaly in maize associated with virus infection. Genetics 54:1287-1296.
Stuber, C. W., and C. S. Levings. 1969. Auxin induction and repression of peroridase isozymes in plant tissue. Crop Sci. 9:000-000.
Ursprung, H., K. D. Smith, W. H. Sofer, and D. T. Sullivan. 1968. Assay systems for the study of gene function. Science 160:1075-1081.
Warner, Robert, R. H. Hageman, J. W. Dudley, and R. J. Lambert. 1969. Inheritance of nitrate reductase activity in Zea mays L. Proc. Nat. Acad. Sci. USA. (in press)

16... DISCUSSION

NEAL F. JENSEN

Cornell University
Ithaca, New York

Dr. Sprague is to be congratulated for his thoughtful analysis and clear exposition of a difficult and neglected area of vital importance to crop improvement. Especially valuable are the many illustrations and references to published work. I found provocative his fine distinction between biochemical genetics and physiological genetics and his preference for the latter where "biosynthetic pathways and the isolation and characterization of enzymes would not be an end in themselves, but would be extended to explore the effects of individual enzymes or of enzyme systems on all aspects of growth, development, and reproductive capacity." Plant breeders, particularly, would applaud the growth of a form of genetics that would show a clear relationship to plant improvement.

One cannot read or hear Dr. Sprague's paper without being led to interesting speculations such as, what happens to an efficient primary gene product that has the misfortune to be associated with less efficient gene products in a multistep process? Or, what is the relation between

hybrid vigor or unusual activity in the seedling stage and end product productivity?

However useless it would be for me to pose as a physiological geneticist I believe I may lay claim legitimately as a plant breeder to an appreciation for this type of research, in fact, I find that in 1968 on no fewer than eight occasions I used physiological ecology as the theme for public talks or espoused its cause in meetings concerned with crop improvement. The committee on biological efficiency of the USDA-State Experiment Station Joint Task Force that last year assessed the research needs of the small grains for the next 10 years gave this subject its highest priority. In addition, the completed task force document carries in its recommendations a statement which I quote in part:

"The yield, quality, physical traits and other characteristics of the cereal varieties grown on millions of USA acres are the result of a continuing interaction between the genotype of the plant and its changing total environment; in fact, they are the result of a sequence of biochemical reactions monitored by enzymes, themselves under the control of genes. This whole area of the physiological ecology of the cereals is not well understood; high yielding varieties have been bred without fully understanding why they are superior in performance. For crop scientists to progress in the future it is essential that favorable plant metabolic processes be recognized and used in variety development and crop production.

"Biological efficiency involves a host of important subject areas but the Task Force wishes to stress the need for research on the basic biological processes, morphological traits, response characteristics and genotype-environment interactions in the cereals so that these traits will become as well known as an useable in plant improvement as are, for example, genes for height, disease resistance or stiff straw."

I want now to give you 2 postulates that in my view emphasize the necessity to expand research in the areas Dr. Sprague has designated as physiological genetics. These are:

1) The rate of progress in plant improvement will decrease with time.

2) Our breeders are exhausting resource materials suitable to present systems and must soon receive supplementary research information of a nature suitable to more complex and sophisticated procedures.

Taking these in order, the first postulate that the rate of progress in plant improvement will decrease with time is another way of saying, "Yes, Virginia, there is a ceiling." Plant improvement in its manifold forms is, after all, finite. Yield is finite. One can, if he wishes, speculate as to what the ultimate yield limit of a given crop might be under the most favorably endowed environment. While we do not need to concern ourselves here with such figures, nor the question of time scale, it is important to recognize during this present period of significant productivity advances that each move to higher yield levels uses up some of the finite potential. The question I raise here is a curious one: can we increase the rate at which we use up this remaining finite resource? We note the quantum jumps being made in wheat and rice yields, for example. While giving proper due to the breeder let us also

be aware of the element of fortune inherent in such successful samplings of our world germplasm resources. In the case of wheat these successes indicate a sizeable as-yet-unexploited variability pool; certain other crops do not show this favorable aspect. A proper topic for discussion by geneticists and plant breeders would be a consideration of possibly better ways to sample and use the variability present in world germplasm collections than our present haphazard and subjective ones.

The second postulate that our breeding methods are exhausting resource materials to the point that supplemental assistance is needed, is based upon knowledge of a characteristic inseparable from the methodology of almost all breeding programs, that of empiricism. As Hageman, Leng, and Dugley pointed out in the paper referred to by Dr. Sprague, "Yield testing in the field is the major selection tool." I mean by the empirical method that plant breeders long ago found that by using standard plot techniques superior genotypes as we define them would be sorted out by the process and identified in the data sheets, almost automatically. Indeed, as we all know, empirical methods have given successful results but now we recognize a deficiency in the method: it adds little to our store of knowledge or understanding as to how the results were obtained and, particularly, why a superior genotype yields and performs as it does. Not knowing why we are unable to identify and use the responsible traits as genetic building blocks in further breeding. This weakness of the empirical method will become more apparent as performance levels are raised. To illustrate from my own work two decades ago it was possible to release a superior wheat variety in New York and then look within the project with confidence for the appearance of its successor; today, with the release of Yorkstar, and projected cumulative yields at levels 60% higher, I am not so sure that empiricism can play the same role. We are seeking for direction for future breeding programs retaining, if possible, the empirical framework. Two avenues, particularly, appear to me to offer promise of help. These are:

1) A modification of breeding methods to increase predictability and odds of successfully reaching objectives, and

2) An increase in our knowledge of the form and function components of productivity. The latter has been the subject of Dr. Sprague's talk.

In conclusion, I have pointed out two characteristics of varietal development for crop improvement, namely, (i) prospects for an eventual decline in the rate of growth of crop productivity, with the suggestion that we reverse conservation concepts and work to exploit this finite resource as rapidly as possible, and (ii) a flaw in the empirical breeding method. Dr. Sprague offers hope that we can supplement our present procedures by adding to the available tools and building blocks of the plant breeder. He is asking that physiologists establish with geneticists and plant breeders the same kind of close working cooperation that has already been in existence for decades with plant pathologists, entomologists, and agronomists. Specifically, he is asking that information be obtained on the influence of alternative states of form or function on plant performance.