COMPARATIVE STUDIES OF SEED ENZYMES OF SPECIES OF GOSSYPIUM

BY POLYACRYLAMIDE AND STARCH GEL ELECTROPHORESIS

by

John Paul Cherry

A Dissertation Submitted to the Faculty of the

COMMITTEE ON GENETICS

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1971
I hereby recommend that this dissertation prepared under my direction by John Paul Cherry entitled COMPARATIVE STUDIES OF SEED ENZYMES OF SPECIES OF GOSSYPIUM BY POLYACRYLAMIDE AND STARCH GEL ELECTROPHORESIS be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy.

Dissertation Director Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.
STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: [Signature]

[Name]
ACKNOWLEDGMENTS

The author is indebted to his advisor, Dr. Frank R. H. Katterman, for his suggestion of the subject of this dissertation and for his tactful guidance and constructive criticisms during the research and writing stages. Similarly, the author wishes to thank Dr. John E. Endrizzi for his suggestions, encouragement, and guidance during this research program. In addition, the author wishes to express his sincere appreciation to Drs. Katterman and Endrizzi for their guidance through his doctoral program not only as faculty members of the Committee on Genetics but also as friends.

Special appreciation goes to the Department of Plant Breeding for financial support in the way of a Research Associateship awarded to the author as well as research facilities and equipment.

The author would like to thank Dr. Robert M. Harris and Dr. Kaoru Matsuda for reading and criticizing this manuscript. Special thanks go to Dr. Albert Siegel for his criticisms and constructive guidance in the writing of this manuscript.

The very capable assistance in the electrophoretic analyses by Miss Vickie L. Day was appreciated. The author gives special thanks to his wife Janet and to Mrs. Patricia Stephens for typing the rough drafts of this manuscript, and to Mrs. J. L. Cude for typing it in excellent final form.
A very special appreciation and gratitude is warranted to my wife Janet, and my parents, Mr. and Mrs. John Cherry, Jr., for their continuous encouragement, understanding, and patience during the course of this entire program.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>x</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>4</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>12</td>
</tr>
<tr>
<td>Species and Varieties Examined</td>
<td>12</td>
</tr>
<tr>
<td>Extraction of Enzymes</td>
<td>15</td>
</tr>
<tr>
<td>Polyacrylamide Gel Electrophoresis</td>
<td>16</td>
</tr>
<tr>
<td>Starch Gel Electrophoresis</td>
<td>16</td>
</tr>
<tr>
<td>Examination of Enzyme Activity</td>
<td>16</td>
</tr>
<tr>
<td>Preparation of Diagrams</td>
<td>19</td>
</tr>
<tr>
<td>RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>Esterase Polymorphism in Natural Populations of Gossypium Thurberi</td>
<td>24</td>
</tr>
<tr>
<td>Analysis of Species and Varieties by Polyacrylamide and Starch Gel Electrophoresis</td>
<td>32</td>
</tr>
<tr>
<td>A Genome Comparison</td>
<td>32</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>32</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>32</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>33</td>
</tr>
<tr>
<td>B Genome Comparison</td>
<td>33</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>33</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>33</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>33</td>
</tr>
<tr>
<td>C Genome Comparison</td>
<td>33</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>33</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>33</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>33</td>
</tr>
<tr>
<td>D Genome Comparison</td>
<td>35</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>35</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>35</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>37</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS—Continued

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>E Genome Comparison</td>
<td>38</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>38</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>39</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>39</td>
</tr>
<tr>
<td>F Genome Comparison</td>
<td>39</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>39</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>39</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>39</td>
</tr>
<tr>
<td>Comparison of the Enzyme Banding Patterns Between the Six Genomes</td>
<td>40</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>40</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>43</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>43</td>
</tr>
<tr>
<td>Analysis of the Natural Allotetraploid Species and Varieties</td>
<td>44</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>44</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>44</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>45</td>
</tr>
<tr>
<td>Comparison of the Three Natural Allotetraploids to Two Genome and Their Synthetic Mixtures</td>
<td>45</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td>45</td>
</tr>
<tr>
<td>Leucine Aminopeptidase Activity</td>
<td>47</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>47</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>50</td>
</tr>
<tr>
<td>Esterase Isozyme Polymorphism in Natural Populations of Gossypium Thurberi</td>
<td>51</td>
</tr>
<tr>
<td>Analysis of the Genome Groups of the Genus Gossypium</td>
<td>53</td>
</tr>
<tr>
<td>The Relationship Between Species in Africa to the Species Removed From This Postulated Center of Origin</td>
<td>60</td>
</tr>
<tr>
<td>Analysis of the Allopolyploids of the Genus Gossypium</td>
<td>62</td>
</tr>
<tr>
<td>Other Chemotaxonomic Studies Concerning the Genus Gossypium</td>
<td>70</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>71</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>74</td>
</tr>
<tr>
<td>Figure</td>
<td>Illustration</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Geographical distribution of the diploid and allo-tetraploid species of the genus <em>Gossypium</em></td>
</tr>
<tr>
<td>2.</td>
<td>Esterase activity: Pairwise comparison of species from within and between genome groups with their synthetic mixtures by polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>3.</td>
<td>Leucine aminopeptidase activity: Pairwise comparison of species from within and between genome groups with their synthetic mixtures by starch gel electrophoresis</td>
</tr>
<tr>
<td>4.</td>
<td>Catalase activity: Pairwise comparison of species from within and between genome groups with their synthetic mixtures by starch gel electrophoresis</td>
</tr>
<tr>
<td>5.</td>
<td>Polyacrylamide gel electrophoretic esterase spectra of different natural populations of <em>G. thurberi</em></td>
</tr>
<tr>
<td>6.</td>
<td>Polyacrylamide gel electrophoretic esterase spectra of four and six varieties of the A₁ and A₂ genomes, respectively, and of two species within the B genome</td>
</tr>
<tr>
<td>7.</td>
<td>Starch gel electrophoretic leucine aminopeptidase spectra of diploid and allotetraploid species of <em>Gossypium</em></td>
</tr>
<tr>
<td>8.</td>
<td>Starch gel electrophoretic catalase spectra of diploid and allotetraploid species of <em>Gossypium</em></td>
</tr>
<tr>
<td>9.</td>
<td>Polyacrylamide gel electrophoretic esterase spectra of five species within the C genome, four species within the E genome, and one species from the F genome</td>
</tr>
<tr>
<td>10.</td>
<td>Polyacrylamide gel electrophoretic esterase spectra of nine species within the D genome</td>
</tr>
<tr>
<td>11.</td>
<td>Polyacrylamide gel electrophoretic esterase spectra of the zymograms occurring in highest frequencies in the diploid and allotetraploid species of <em>Gossypium</em></td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>12.</td>
<td>Polyacrylamide gel electrophoretic esterase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids</td>
</tr>
<tr>
<td>13.</td>
<td>Starch gel electrophoretic leucine aminopeptidase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids</td>
</tr>
<tr>
<td>14.</td>
<td>Starch gel electrophoretic catalase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids</td>
</tr>
<tr>
<td>15.</td>
<td>Polyacrylamide gel electrophoretic esterase spectra of a comparison between G. hirsutum var. Acala 44-10-1, 2(AD)₁, G. sturtianum var. sturtianum (C₁), their synthetic mixture, and the synthetic triploid and colchicine-induced hexaploid, Experimental 6x-3</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Species of the genus <em>Gossypium</em></td>
<td>13</td>
</tr>
<tr>
<td>2.</td>
<td>Seed weights, and amount and percent of tris-glycine soluble protein estimated per seed of the diploid species and varieties</td>
<td>17</td>
</tr>
<tr>
<td>3.</td>
<td>Seed weights, and amount and percent of tris-glycine soluble protein estimated per seed of the allopolyploid species and varieties</td>
<td>18</td>
</tr>
<tr>
<td>4.</td>
<td>Frequency of occurrence of esterase patterns in zymograms A-F of the natural populations of <em>Gossypium thurberi</em></td>
<td>26</td>
</tr>
</tbody>
</table>
ABSTRACT

Classical techniques including morphological, geographical, cytological, and hybridization analyses have distinguished the diploid species of the genus *Gossypium* into six genome groups (A-F). The genomes of the three natural allotetraploid species were labeled 2(AD)n. Further support to this classification was presented by means of polyacrylamide and starch gel electrophoresis of aqueous extracts of dormant seed esterase, leucine aminopeptidase, and catalase of 29 species and 29 varieties (including diploids and synthetic and natural allopolyploids) of the genus *Gossypium*.

Much isozyme variation (qualitative and quantitative) was observed for esterase activity within as well as between the species of the different genome groups. Therefore, to predict a possible phylogenetic relationship between species, intraspecific enzyme polymorphism was evaluated before interspecific comparisons were undertaken. The within species polymorphism as shown for the esterases was not observed for the leucine aminopeptidase and catalase isozyme patterns. However, in support of the classification of the genus *Gossypium*, species and/or varieties within each genome group exhibited zymograms for the three enzymes that were more similar to one another than to members of the other genomes.

Species with esterase, leucine aminopeptidase, and catalase zymograms similar to that produced by the *A*1 and *D*5 genomes were indicated to be the most likely ancestors of the natural allotetraploids.
(G. tomentosum, G. barbadense, and G. hirsutum). This observation was based on a comparison of the additive zymograms in the synthetic mixtures of the diploid species to the isozyme patterns of the natural allotetraploids. In addition, these analyses of the three enzymes showed quantitative and qualitative isozyme variations between the three natural allotetraploids when compared to the zymograms of the synthetic mixtures of their alleged parents. These isozyme variations were not observed in the comparisons of the recently synthesized allopolyploids. Diploidization and a recently proposed regulation mechanism as possible evolutionary processes for higher organisms were discussed in relation to these isozyme differences observed in the natural allotetraploids.
INTRODUCTION

During the past decade, the techniques of chemotaxonomy have added a new dimension to the classical methods of geographical, morphological, cytological, and hybridization analyses generally used for phylogenetic studies. The methods of polyacrylamide and starch gel electrophoresis have been especially useful in this respect.

Amino acid changes in a protein, due to gene mutation, can result in an altered migration rate in an electric field. Therefore, since species differ genetically at many gene loci, the individuality of each plant species can usually be observed as a protein and/or enzyme electrophoretic banding pattern.

Isozymes are forms of enzymes which catalyze similar reactions but differ in composition. The differences between isozymes have been found to be beneficial to organisms by providing alternate enzyme species which may function better during the different stages of development. Also, the presence of a number of genes and/or alleles coding for the same type of enzyme activity can provide a form of biochemical adaptability to different environments.

Since the early 1900's much has been accomplished with the taxonomic classification of the genus Gossypium. The genus is comprised of about 30 diploid and three natural allotetraploid species. The diploid species are divided into six genome groups, A through F. The other three species are allotetraploids containing genomes A and D. The
species of the genus *Gossypium* that are presently obtainable are listed in Table 1 (see p. 13) and their geographical distribution is shown in Figure 1 (see p. 14). These data have been fully discussed by Beasley (1942), Hutchinson, Silow, and Stephens (1947), Phillips (1963), Fryxell (1965a) and Cherry, Katterman, and Endrizzi (1970).

The availability of the species within genome groups enables one to make a comparison of protein and isozyme composition, for selected enzyme systems, within and between the genome groups for the purpose of complementing the data concerning the taxonomic classification of the genus.

Cherry, Katterman, and Endrizzi (1970) performed an extensive analysis on the genus *Gossypium* by means of polyacrylamide gel electrophoresis of proteins obtained from dormant seed. A comparison of the protein banding patterns for species within and between genomes gave supportive data to the present classification of *Gossypium* species and to the origin of the natural allotetraploids.

With these points in mind, an analysis of the isozyme composition of several specific enzyme systems, using the techniques of polyacrylamide and starch gel electrophoresis, was performed on 29 species and 29 varieties (including diploids and synthetic and natural allopolyploids) in the genus *Gossypium*. The isozyme systems studied were esterase, leucine aminopeptidase, and catalase present in the dormant seeds.

The present work revealed several different esterase zymograms within the species of the different genome groups. Therefore, to predict the possible taxonomic relationship between the species with
regard to a given isozyme pattern, intraspecific variation was evaluated before interspecific analyses were undertaken. In contrast to the esterase activity, no variable intraspecific isozyme patterns were observed for the leucine aminopeptidase and catalase activity.
Polyacrylamide and starch gel electrophoresis of seed enzymes offers a biochemical approach to the evolutionary aspects of plant speciation. The background and theory of polyacrylamide gel electrophoresis were thoroughly discussed by Ornstein (1964), and the method and application of the technique to analyze and compare serum proteins were presented by Davis (1964). Smithies (1955) introduced zone electrophoresis in starch gels as a technique of high resolving power for separating different mixtures of proteins. Hunter and Møller (1957) combined this technique with histochemical staining procedures for the demonstration of esterases. In addition, this latter study revealed a vast multiplicity of electrophoretically distinct molecular forms with esterase activity.

The term "isozyme" was proposed by Møller and Møller (1959) to refer to enzymatically active proteins that are separated by electrophoretic techniques which catalyze one and the same biochemical reaction. Møller (1968) proposed modifying the term isozyme with terms such as allelic, nonallelic, homopolymeric, conformational, hybrid, and conjugated. Recently, Shaw (1969) classified isozymes into (a) primary types which include distinct molecular entities produced from different genetic sites, and (b) secondary types which result from a significant alteration in structure of a single polypeptide species and which may be in vitro artifacts. In addition, Scandalios (1969) has employed the term isozyme to define the heterozygous state of genetically variant enzymes.
The principal types of molecular multiplicity known to generate isozyme patterns were reviewed by Markert and Whitt (1968). They include the following: (a) different polypeptides encoded by allelic and nonallelic genes, (b) polymers of various sizes, (c) homopolymers and heteropolymers, (d) polypeptides secondarily modified in various ways, and (e) different conformations produced by permutations of polymer subunits or alternate tertiary and quaternary configurations of proteins. Such molecular diversity indicated that organisms have utilized both genetic and epigenetic mechanisms to evolve molecular properties of enzymes to fit special metabolic requirements.

Electrophoretic investigations of genetic control of enzymes in plants were reviewed by Scandalios (1969). These studies revealed a multiplicity of alleles or genes carrying information for a specific type of enzyme activity. For example, crosses between several lines of maize with different catalase phenotypes revealed six alleles, whereas leucine aminopeptidase isozymes were found to be controlled by two separate genetic loci. Furthermore, it was noted that different isozymes were present in different tissues and in extracts from different stages of development.

Another aspect of controlled enzyme synthesis in plants was discovered by Williamson, Kleese, and Snyder (1968) who found seedling esterase isozymes to vary within and between specific varieties of *Avena sativa*. The variety Orbit, believed to be widely adapted, contained more variability than Putnam 61, a narrowly adapted variety. They
suggested that the adaptability of a variety may be linked to isozyme variations.

The studies of genetically controlled isozymes have provided an additional means of analyzing taxonomic classifications that have already been established by the well-known classical methods. It has been shown, however, that several problems can occur during the course of such investigations. These problems are discussed below.

Numerous electrophoretic studies have been conducted with the dehydrogenase and hydrolase isozymes from different tissues (root, tuber, pod, seed, germinated seeds, coleoptile, leaf, and fruit) from a wide range of plant systems. The investigations included those of Scandalios (1964) with various tissues of Zea mays; Schwartz et al. (1964) with Cucurbitaceae; Macko, Honold, and Stahmann (1967) and Bhatia and Nilson (1969) with Triticum aestivum; Makinen (1968) with Allium cepa; Upadhya and Yee (1968) with Hordeum vulgare; Scandalios and Espiritu (1969) with Pisum sativum; and Hall et al. (1969) with different types of crop ornamental plants. The general conclusion from these studies was that isozyme patterns varied within and between tissues of the same plant and were dependent upon the ontogenetic stage of development. These observations suggested that when comparing species for taxonomic purposes, care should be taken that the tissues used be of the same stage of development.

An example of the problem that can arise as a result of tissue variation was noted when Hart and Bhatia (1967) compared the esterase, leucine aminopeptidase, and peroxidase activity in leaf extracts of six
species of *Nicotiana*, including four genotypes of *N. rustica* and two varieties of *N. tabacum*; and in addition three ontogenetic stages of leaf development in one variety of *N. tabacum*. Although the four genotypes and two varieties displayed significant differences in banding patterns, variability to an equal extent was observed between species and between the three stages of ontogenetic development. These results indicated that leaf tissue is not an ideal material for making intra- and interspecies comparisons of isozymes.

In addition to the above problems, Scogin (1969) demonstrated that within a single tissue of a given species in a defined physiological state, there may exist a genetically based polymorphism. This work included an examination of isozyme patterns of natural populations of three species of *Baptisia* (Leguminosae). Therefore, to predict the possible taxonomic or physiological relationships from isozyme patterns, intraspecific variation should be evaluated before interspecific analyses are made. These conclusions were similarly drawn by Thurman et al. (1967), examining formic and glutamic dehydrogenase zymograms of dry seeds from species in the family of Fabaceae, and by West and Garber (1967a), studying esterase and leucine aminopeptidase activity of cotyledons from germinating seedlings of species of *Phaseolus*.

Similarly, Shahi, Morishima, and Oka (1969) made a survey of the within-and-between-species isozyme variations of peroxidase, acid phosphatase, and esterase from the leaf tissue of *Oryza*. Interestingly, variability within species was greater than between species. This
phenomena made it difficult to identify species by zymograms. Arrangement of strains according to their isozyme banding similarities, however, showed that wild species with wide geographical distributions generally had a large amount of variation, whereas localized wild as well as cultivated species exhibited a limited variation.

With these limitations in mind, the following are some successful examples of isozyme analyses in which different plant tissues were utilized. Wennstrom and Garber (1965) examined aqueous and acetone extracts of germinating seeds, flowers, and young leaves of 12 species and one interspecific hybrid in the genus Collinsia. Their study showed that these species contained esterases and acid phosphatases that varied between the different tissues of the plants. However, all tested species gave the same pattern when one plant part and one method of extraction were used.

A survey of the esterase and leucine aminopeptidase activity in crude extracts from cotyledons of germinating seedlings of 15 species of Phaseolus was completed by West and Garber (1967a). This analysis showed that each of the 15 species could be distinguished by comparing the isozyme banding patterns. West and Garber (1967b) presented additional results from a study of the esterase and leucine aminopeptidase activity in interspecific hybrids of Phaseolus and the progeny of the backcross of the $F_1$ to the parents. Upon comparison of amphidiploids to their parent species, it was found that both enzyme systems were monogenic in origin and that the two amphidiploids exhibited all of the sites of esterase and leucine aminopeptidase activity observed in their
respective parent species. These comparisons suggested that in addition to species classification, the zymogram may be a valuable tool for investigations concerned with introgressive hybridization and with the origin of polyploid species.

Recently Eguchi and Matsui (1969a,b) examined esterase zymograms from cotyledonary tissue of *Brassica* and *Cucurbita* seedlings. The results of these studies gave further support to the classical taxonomic and phylogenetic classification of the two genera.

There are a number of researchers who believe that enzymes from seed or other dormant tissues of plants represent a more stable reflection of the genome state in a given species than that obtained from developing seedlings. Vaughan and Waite (1967a,b) examined seed beta-glucosidase, beta-galactosidase, and esterase activity from a number of the former. Results from the analysis of the first two enzymes agreed with the already known phylogenetic relationships of the diploid species. All three enzymes gave good supportive evidence as to the possible parents of the amphidiploids.

The variation and distribution of esterase isozymes in *Solanum* species and cultivar tubers were examined by Desborough and Peloquin (1967). Esterases obtained from tubers of wild species, interspecific hybrids, haploids, haploid-species hybrids, and selfs of cultivars revealed a diversity of patterns. No simple or direct taxonomic relationship between esterase patterns and a series of species was observed. Aqueous seed extracts of only six of the species of *Cucurbita*, including five cultivars of two species, and one species hybrid were examined
for their esterase and leucine aminopeptidase isozyme patterns by Wall (1969). The data from this study and that with Solanum suggested that more extensive analyses, including isozyme patterns of many enzymes and species, respectively, were needed to solve the evolutionary and systematic problems of the different genera.

Crowden, Harborne, and Heywood (1969) emphasized the significance of examining a sufficient number of species to show the true potential of seed proteins and enzymes as general taxonomic characters. Here, extracts from 174 samples of seed, covering 99 species and 39 genera, with representatives from all 8 tribes of the subfamily Apioideae in the family Umbelliferae were examined electrophoretically. The enzymes examined were esterase, peroxidase, catalase, and amylase. This investigation revealed distinct differences at the tribal, generic, and species level similar to that of the taxonomic data.

In summary, polyacrylamide and starch gel electrophoresis of enzymes within different plant tissues offers a biochemical approach to the taxonomic and phylogenetic aspects of plant speciation. A large amount of molecular diversity and multiplicity of enzymes in the form of isozymes is present and is under genetic control. Studies have shown that these isozyme patterns vary within and between tissues of the same plant depending upon the ontogenetic stage of development. Thus, when comparing different plants for taxonomic purposes, care must be taken to insure that the tissues used are at the same stage of development. Further work demonstrated that much intra- as well as interspecific variation exists. Therefore, intraspecific variation should be evaluated before interspecific analyses are made. In addition, experiments should
be planned whereby a sufficient number of enzymes and species of a genus are examined.

In view of these observations, many workers have undertaken electrophoretic analyses of enzymes to further elucidate or confirm taxonomic relations of many species within and between a number of genera obtained previously by the well-known classical methods.
MATERIALS AND METHODS

Species and Varieties Examined

The wild diploid and natural allotetraploid species analyzed are presented in Table 1 along with their respective genomic symbols and geographical locations. The following types were also included in the study but are not listed in Table 1: three varieties of *G. herbaceum*, the wild type, var. africanum (G304), and two cultivated types, AG152 and AG153; five varieties of *G. arboreum*, AG109, G25, G10, G266, G24; a synthetic allotriploid (*G. hirsutum*, var. Acala 44-10-1 x *G. sturtianum* Willis) and its colchicine produced hexaploid (Experimental 6X-3); four varieties of *G. barbadense*, Pima S x P, Pima S-2, Menoufi, and Sea Island; and 15 varieties of *G. hirsutum*, Acala 44-10-1, Acala 1517A, Stoneville 7A, Mexican Big Boll, Hibred, Paymaster 54B, Kechi, Lockett 4789, CB3051 (Yugoslavian strain), Macha, Clevewilt, King 82, Contextum, Rowden, and Hopi Sacaton (see Figure 1 for *Gossypium* distribution).

Seeds for analysis of a number of species and types were collected from plants grown at The University of Arizona. Also, other seed were supplied through the courtesy of the Crops Research Division of the United States Department of Agriculture and the Cotton Research Stations at Tempe, Arizona, P. A. Fryxell at College Station, Texas, and L. S. Stith and H. Muramoto of the Department of Plant Breeding, The University of Arizona, Tucson.
Table 1. Species of the genus *Gossypium*.

<table>
<thead>
<tr>
<th>Genome</th>
<th>Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Diploid (2n=26)</strong></td>
<td></td>
</tr>
<tr>
<td>A₁</td>
<td><em>G. herbaceum</em> L.</td>
<td>Old World Cultigen</td>
</tr>
<tr>
<td>A₂</td>
<td><em>G. arboreum</em> L.</td>
<td>Old World Cultigen</td>
</tr>
<tr>
<td>B₁</td>
<td><em>G. anomalum</em> Wawr. &amp; Peyr.</td>
<td>Africa</td>
</tr>
<tr>
<td>B₂</td>
<td><em>G. triphyllum</em> (Harv. ex Harv. &amp; Sond.) Hochr.</td>
<td>Africa</td>
</tr>
<tr>
<td>C₁</td>
<td><em>G. sturtianum</em> var. sturtianum Willis</td>
<td>Australia</td>
</tr>
<tr>
<td>C₁-n</td>
<td><em>G. sturtianum</em> var. <em>nandewarense</em> (Der.) Fryx.</td>
<td>Australia</td>
</tr>
<tr>
<td>C₂</td>
<td><em>G. robinsonii</em> F. Muell.</td>
<td>Australia</td>
</tr>
<tr>
<td>C₃</td>
<td><em>G. australe</em> F. Muell.</td>
<td>Australia</td>
</tr>
<tr>
<td>C₄</td>
<td><em>G. bickii</em> Prokh.</td>
<td>Australia</td>
</tr>
<tr>
<td>D₁</td>
<td><em>G. thurberi</em> Tod.</td>
<td>Mexico, Arizona</td>
</tr>
<tr>
<td>D₂₁</td>
<td><em>G. armourianum</em> Kearn.</td>
<td>Mexico</td>
</tr>
<tr>
<td>D₂-2</td>
<td><em>G. harknessii</em> Brandg.</td>
<td>Mexico</td>
</tr>
<tr>
<td>D₃-d</td>
<td><em>G. davidsonii</em> Kell.</td>
<td>Mexico</td>
</tr>
<tr>
<td>D₃-k</td>
<td><em>G. klotzschianum</em> Andress.</td>
<td>Galapagos</td>
</tr>
<tr>
<td>D₄</td>
<td><em>G. aridum</em> (Rose &amp; Standl.) Skov.</td>
<td>Mexico</td>
</tr>
<tr>
<td>D₅</td>
<td><em>G. raimondii</em> (Ulbr.)</td>
<td>Peru</td>
</tr>
<tr>
<td>D₆</td>
<td><em>G. gossypioides</em> (Ulbr.) Standl.</td>
<td>Mexico</td>
</tr>
<tr>
<td>D₇</td>
<td><em>G. lobatum</em> Gentry</td>
<td>Mexico</td>
</tr>
<tr>
<td>E₁</td>
<td><em>G. stocksii</em> Mast. ex Hook.</td>
<td>Arabia</td>
</tr>
<tr>
<td>E₂</td>
<td><em>G. somalense</em> (Gurke) Hutch.</td>
<td>Africa</td>
</tr>
<tr>
<td>E₃</td>
<td><em>G. areysianum</em> (Defl.) Hutch.</td>
<td>Arabia</td>
</tr>
<tr>
<td>E₄</td>
<td><em>G. incanum</em> (Schwartz) Hillc.</td>
<td>Arabia</td>
</tr>
<tr>
<td>F₁</td>
<td><em>G. longicalyx</em> Hutch. &amp; Lee</td>
<td>Africa</td>
</tr>
</tbody>
</table>

|        | **Tetraploid (4n=52)** | |
| (AD)₁  | *G. hirsutum* L. | New World Cultigen |
| (AD)₂  | *G. barbadense* L. | New World Cultigen |
| (AD)₃  | *G. tomentosum* Nutt. ex Seem. | Hawaii |

Figure 1. Geographical distribution of the diploid and allotetraploid species of the genus Gossypium.
Gossypium thurberi seeds for analysis were collected from a number of plants at each of four population sites distributed north, east, south, and west of Tucson, Arizona. These sites include: Santa Catalina Mts., at Molino Basin (north); Rincon Mts., at the Madrona Ranger Station area (east); Santa Rita Mts., at Madera Canyon (south); and the Baboquivari Mts., on the road to Kitt Peak (west). Hanson (1923) has described the plant *G. thurberi* along with its habitats and locations within Arizona.

**Extraction of Enzymes**

Twenty-four seeds of each species were used for the esterase analysis by polyacrylamide gel electrophoresis. For the study of esterase variability in the populations of *G. thurberi*, 268 seeds were used (Table 4, p. 26). The analysis of leucine aminopeptidase and catalase activity on starch gel electrophoresis involved 12 seeds from each species.

All procedures were carried out at 0-4°C. For each species examined, replicated studies involving in each case one seed that was ground by means of a mortar and pestle for each extraction in 0.4-0.9 ml of tris-glycine buffer (0.1 M; pH 8.3) were conducted. The crude extracts were partially purified by centrifugation for 10 minutes at 4300 g in a Sorvall refrigerated centrifuge. The supernatant fraction was then submitted to electrophoresis to determine their isozyme make-up. The banding patterns from these samples were also assayed at three dilutions in which the technique for examination of proteins by Cherry, Katterman, and Endrizzi (1970) was utilized. This latter examination
allowed for the analysis of each gel to determine a relative estimate of their qualitative and quantitative make-up. Tables 2 and 3 show the protein concentration per seed for each species and variety examined. The extraction procedure for this protein analysis was that of Cherry, Katterman, and Endrizzi (1970). The crude extracts were analyzed for protein content using the technique of Lowry et al. (1951), using bovine serum albumin as a standard.

**Polyacrylamide Gel Electrophoresis**

A Canalco Model 1200 Bath electrophoretic apparatus and a Beckman Model Rd-2 Duostat power source were used. The methods of preparation of the gel and conditions of electrophoresis were similar to Steward, Lyndon, and Barber (1965), with the modifications of Cherry, Katterman, and Endrizzi (1970).

**Starch Gel Electrophoresis**

A homemade apparatus was constructed following the procedures of Brewbaker et al. (1968). A Heathkit IP-32 power supply unit was used. The methods of preparation of the starch gel and the analysis of the samples were also similar to the techniques developed by these authors.

**Examination of Enzyme Activity**

Esterases were stained using the technique of Johnson et al. (1966) with the following modifications: (1) enzyme activity was allowed to continue for one hour at room temperature and (2) fast blue RR was used as a stain. The excess stain on the gels was removed by overnight destaining in a solution of methanol:acetic acid:water (50:10:50,
Table 2. Seed weights, and amount and percent of tris-glycine soluble protein estimated per seed of the diploid species and varieties.

<table>
<thead>
<tr>
<th>Species &amp; varieties</th>
<th>Seed weight (gm/seed)</th>
<th>Protein content (gm/seed)</th>
<th>Protein content (%/seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. herbaceum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. G304</td>
<td>.048</td>
<td>.0043</td>
<td>8.9</td>
</tr>
<tr>
<td>var. AG152</td>
<td>.084</td>
<td>.0065</td>
<td>7.7</td>
</tr>
<tr>
<td>var. AG153</td>
<td>.066</td>
<td>.0061</td>
<td>9.3</td>
</tr>
<tr>
<td>Average</td>
<td>.066</td>
<td>.0056</td>
<td>8.6</td>
</tr>
<tr>
<td>G. arboreum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. G10</td>
<td>.090</td>
<td>.0074</td>
<td>8.2</td>
</tr>
<tr>
<td>var. AG109</td>
<td>.048</td>
<td>.0043</td>
<td>8.9</td>
</tr>
<tr>
<td>var. G25</td>
<td>.047</td>
<td>.0041</td>
<td>8.7</td>
</tr>
<tr>
<td>var. G266</td>
<td>.060</td>
<td>.0050</td>
<td>8.3</td>
</tr>
<tr>
<td>var. G24</td>
<td>.061</td>
<td>.0049</td>
<td>8.0</td>
</tr>
<tr>
<td>Average</td>
<td>.061</td>
<td>.0051</td>
<td>8.4</td>
</tr>
<tr>
<td>G. anomalum</td>
<td>.019</td>
<td>.0020</td>
<td>10.4</td>
</tr>
<tr>
<td>G. triphyllum</td>
<td>.020</td>
<td>.0024</td>
<td>12.4</td>
</tr>
<tr>
<td>Average</td>
<td>.020</td>
<td>.0022</td>
<td>11.4</td>
</tr>
<tr>
<td>G. sturtianum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. sturtianum</td>
<td>.009</td>
<td>.0010</td>
<td>10.7</td>
</tr>
<tr>
<td>var. nandewarense</td>
<td>.009</td>
<td>.0010</td>
<td>10.7</td>
</tr>
<tr>
<td>G. robinsonii</td>
<td>.009</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G. australis</td>
<td>.008</td>
<td>.0009</td>
<td>11.3</td>
</tr>
<tr>
<td>G. bickii</td>
<td>.010</td>
<td>.0009</td>
<td>8.8</td>
</tr>
<tr>
<td>Average</td>
<td>.009</td>
<td>.0010</td>
<td>10.4</td>
</tr>
<tr>
<td>G. stocksii</td>
<td>.019</td>
<td>.0020</td>
<td>10.5</td>
</tr>
<tr>
<td>G. somalense</td>
<td>.015</td>
<td>.0018</td>
<td>11.6</td>
</tr>
<tr>
<td>G. areysianum</td>
<td>.018</td>
<td>.0023</td>
<td>12.7</td>
</tr>
<tr>
<td>G. incanum</td>
<td>.018</td>
<td>.0023</td>
<td>12.5</td>
</tr>
<tr>
<td>Average</td>
<td>.018</td>
<td>.0021</td>
<td>11.8</td>
</tr>
<tr>
<td>G. longicalyx</td>
<td>.028</td>
<td>.0034</td>
<td>12.2</td>
</tr>
<tr>
<td>G. thurberi</td>
<td>.026</td>
<td>.0030</td>
<td>11.6</td>
</tr>
<tr>
<td>G. armorianum</td>
<td>.061</td>
<td>.0042</td>
<td>6.9</td>
</tr>
<tr>
<td>G. harknessi</td>
<td>.052</td>
<td>.0043</td>
<td>8.2</td>
</tr>
<tr>
<td>G. klotzschianum</td>
<td>.038</td>
<td>.0027</td>
<td>7.1</td>
</tr>
<tr>
<td>G. davidsonii</td>
<td>.042</td>
<td>.0034</td>
<td>8.1</td>
</tr>
<tr>
<td>G. aridum</td>
<td>-</td>
<td>.0018</td>
<td>-</td>
</tr>
<tr>
<td>G. raimondii</td>
<td>.031</td>
<td>.0035</td>
<td>11.4</td>
</tr>
<tr>
<td>G. gossypioides</td>
<td>.029</td>
<td>.0030</td>
<td>10.5</td>
</tr>
<tr>
<td>G. lobatum</td>
<td>.021</td>
<td>.0029</td>
<td>13.9</td>
</tr>
<tr>
<td>Average</td>
<td>.038</td>
<td>.0032</td>
<td>9.7</td>
</tr>
</tbody>
</table>
Table 3. Seed weights, and amount and percent of tris-glycine soluble protein estimated per seed of the allopolyploid species and varieties.

<table>
<thead>
<tr>
<th>Species &amp; varieties</th>
<th>Seed weight (gm/seed)</th>
<th>Protein content (gm/seed)</th>
<th>Protein content (%/seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. tomentosum</td>
<td>.043</td>
<td>.0042</td>
<td>9.7</td>
</tr>
<tr>
<td>G. barbadense</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. Pima SxP</td>
<td>.134</td>
<td>.0092</td>
<td>6.9</td>
</tr>
<tr>
<td>var. Pima S-2</td>
<td>.118</td>
<td>.0088</td>
<td>7.5</td>
</tr>
<tr>
<td>var. Menoufi</td>
<td>.106</td>
<td>.0074</td>
<td>7.0</td>
</tr>
<tr>
<td>var. Sea Island</td>
<td>.129</td>
<td>.0084</td>
<td>6.5</td>
</tr>
<tr>
<td>Average</td>
<td>.122</td>
<td>.0085</td>
<td>7.0</td>
</tr>
<tr>
<td>G. hirsutum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. Acala 44-10-1</td>
<td>.133</td>
<td>.0092</td>
<td>7.0</td>
</tr>
<tr>
<td>var. Acala 1517A</td>
<td>.133</td>
<td>.0092</td>
<td>7.0</td>
</tr>
<tr>
<td>var. Stoneville 7A</td>
<td>.099</td>
<td>.0084</td>
<td>8.5</td>
</tr>
<tr>
<td>var. Mexican Big Boll</td>
<td>.130</td>
<td>.0104</td>
<td>8.0</td>
</tr>
<tr>
<td>var. Hibred</td>
<td>.138</td>
<td>.0104</td>
<td>7.5</td>
</tr>
<tr>
<td>var. Paymaster 5B</td>
<td>.111</td>
<td>.0070</td>
<td>6.3</td>
</tr>
<tr>
<td>var. Kechi</td>
<td>.088</td>
<td>.0060</td>
<td>6.8</td>
</tr>
<tr>
<td>var. Lockett</td>
<td>.110</td>
<td>.0072</td>
<td>6.6</td>
</tr>
<tr>
<td>var. CB 3051</td>
<td>.100</td>
<td>.0084</td>
<td>8.4</td>
</tr>
<tr>
<td>var. Macha</td>
<td>.124</td>
<td>.0088</td>
<td>7.1</td>
</tr>
<tr>
<td>var. Clevewilt</td>
<td>.096</td>
<td>.0064</td>
<td>6.7</td>
</tr>
<tr>
<td>var. King 82</td>
<td>.098</td>
<td>.0080</td>
<td>8.2</td>
</tr>
<tr>
<td>var. Contextum</td>
<td>.076</td>
<td>.0076</td>
<td>9.9</td>
</tr>
<tr>
<td>var. Hopi Sacaton</td>
<td>.090</td>
<td>.0076</td>
<td>8.5</td>
</tr>
<tr>
<td>var. Rowden</td>
<td>.123</td>
<td>.0104</td>
<td>8.3</td>
</tr>
<tr>
<td>Average</td>
<td>.110</td>
<td>.0083</td>
<td>7.7</td>
</tr>
<tr>
<td>AZ239</td>
<td>.062</td>
<td>.0062</td>
<td>9.9</td>
</tr>
<tr>
<td>Triploid</td>
<td>.063</td>
<td>.0059</td>
<td>9.4</td>
</tr>
<tr>
<td>Hexaploid</td>
<td>.063</td>
<td>.0041</td>
<td>6.6</td>
</tr>
</tbody>
</table>
v/v), followed by storage of the gels in 50% methanol. The staining procedures for leucine aminopeptidase and catalase were those of Brewbaker et al. (1968).

**Preparation of Diagrams**

The gels were photographed in Petri Dishes containing 50% methanol, on a diffuse white background which was illuminated from beneath with a fluorescent lamp. All photographs were taken in bright sunlight. The photographed gels were increased in size to 10.5 cm. The drawings included in this dissertation were then made directly from these photographs.

The bands of different species were paired according to the marker band and the origin. In addition, the exact position of the bands within the zymograms was further determined from comparison of the additive banding patterns between species in synthetic mixtures (Figures 2-4). For example, in Figure 2 the esterase zymograms of *G. herbaceum* var. africanum (G304) contains an overall staining pattern of minor bands (light staining bands), whereas *G. armourianum* has four distinct major bands (heavy staining bands). In G304, bands are located at 5.8 cm, 6.3 cm, and 7 cm. These bands are not present in *G. armourianum*. Both individuals show esterase activity in regions 7.6-7.7 cm and 8.7-9.2 cm with slight differences in mobility. In addition, *G. armourianum* has a major band at 8.3 cm not observed in G304. The synthetic mixture of these two individuals is an identical replicate of the two zymograms. This comparison enables one to position the specific bands within the two genomes, A and D. Comparison of a number of species from within and
Figure 2. Esterase activity: Pairwise comparison of species from within and between genome groups with their synthetic mixtures by polyacrylamide gel electrophoresis.

G. herbaceum var. africanum equals var. G304.
Figure 3. Leucine aminopeptidase activity: Pairwise comparison of species from within and between genome groups with their synthetic mixtures by starch gel electrophoresis.

*G. herbaceum* var. africanum equals var. G304.
Figure 4. Catalase activity: Pairwise comparison of species from within and between genome groups with their synthetic mixtures by starch gel electrophoresis.

G. herbaceum var. africanum equals var. G304.
between genomes has given an overall view of the mobility of the iso-
zymes. Similarly, comparisons of leucine aminopeptidase and catalase
are presented (Figures 3 and 4). In all cases the synthetic mixture
produces an additive zymogram of the two species compared.
RESULTS

Esterase Polymorphism in Natural Populations of *Gossypium Thurberi*

The isozyme polymorphism in four natural populations of *G. thurberi* for seed esterase is shown in Figure 5. Six different zymograms (A–F) are observed for the samples of seed analyzed. Within the interval 6.5–9.2 cm, distinct variation (quantitative and qualitative) of bands occur. With the exception of a band at 8 cm, which is present in all cases, zymograms B, D, and F contain a band at 7.3 cm, and F has a band at 6.9 cm. In addition, band 8.6 cm is located in zymograms C, D, E, and F; and E contains a band at 9.2 cm. A band at 6.5 cm, present in zymograms A–D, completes the banding pattern of this variable region.

A comparison of the relative frequencies of seed expressing a specific zymogram from each of the four populations analyzed is shown in Table 4. In general, zymograms A–D occur much more frequently in the natural populations than do zymograms E and F. Similarly, such variation is observed within a large number of the species studied from the different genomes. This variation and the frequency of occurrence of the different zymograms are given in Figures 6, 9, and 10. The esterase data suggest that isozyme variation may be expressed both within and between populations of species. It is also noted that no variables of leucine aminopeptidase and catalase are observed within species of the different genomes (Figures 7 and 8).
Figure 5. Polyacrylamide gel electrophoretic esterase spectra of different natural populations of G. thurberi.
Table 4. Frequency of occurrence of esterase patterns in zymograms A-F of the natural populations of *Gossypium thurberi*.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total seeds analyzed</th>
<th>Zymograms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Santa Catalina Mts.</td>
<td>78</td>
<td>0.718</td>
</tr>
<tr>
<td>Rincon Mts.</td>
<td>72</td>
<td>0.125</td>
</tr>
<tr>
<td>Santa Rita Mts.</td>
<td>72</td>
<td>0.805</td>
</tr>
<tr>
<td>Baboquivari Mts.</td>
<td>45</td>
<td>0.175</td>
</tr>
<tr>
<td>Totals</td>
<td>268</td>
<td>0.488</td>
</tr>
</tbody>
</table>
Figure 6. Polyacrylamide gel electrophoretic esterase spectra of four and six varieties of the $A_1$ and $A_2$ genomes, respectively, and of two species within the B genome. Included are the number of seeds examined and the frequencies of the gels.
Figure 7. Starch gel electrophoretic leucine aminopeptidase spectra of diploid and allotetraploid species of Gossypium.
Figure 3. Starch gel electrophoretic catalase spectra of diploid and all tetraploid species of Gossypium.
Figure 9. Polyacrylamide gel electrophoretic esterase spectra of five species within the C genome, four species within the E genome, and one species from the F genome.

Included are the number of seeds examined and the frequencies of the gels.
Figure 10. Polyacrylamide gel electrophoretic esterase spectra of nine species within the D genome.

Included are the number of seeds examined and the frequencies of the gels.
Analysis of Species and Varieties by Polyacrylamide and Starch Gel Electrophoresis

A Genome Comparison

Esterase Activity. Figure 6 shows that the varieties of G. herbaceum and G. arboreum produce zymograms containing all minor bands. Two varieties of the A genome, AG152 ($A_1$) and G10 ($A_2$) form two different zymograms each, while the other varieties produce one. The three varieties of G. herbaceum contain bands at 6.3 cm, 7 cm, and 8.9 cm. Except for the zymograms of AG152 with a lower frequency, the three varieties contain a band at 5.8 cm. Variety africanum (G304) does not have bands 7.5 cm and 8.6 cm as in the other two varieties. Band 7.7 cm, observed in varieties G304 and AG153, is not present in AG152. Africanum and the low frequency gel of AG152 seem to contain the simplest banding patterns while AG153 is the most complex in this respect within the $A_1$ genome.

The gel patterns of the varieties of G. arboreum are very similar to each other. Varieties G25, G266, G24, and G10 produce zymograms which are identical and contain the same group of bands present in the $A_1$ genome. The low frequency zymogram of variety G10 compares closely to that of variety AG109 in that both patterns lack bands at 7 cm and 7.7 cm present in the other zymograms of the $A_2$ genome. Overall, africanum ($A_1$), the only wild variety of both species, AG109 ($A_2$) and the lower frequency zymograms of AG152 ($A_1$) and G10 ($A_2$) tend to produce the simplest isozyme patterns.

Leucine Aminopeptidase Activity. All varieties of both species contain the same banding patterns (Figure 7). A large major band is
located at 5.7 cm and is followed by a major band of slower mobility and lower activity at 5.2 cm.

**Catalase Activity.** Two major catalase bands are observed in all varieties of the A genome species (Figure 8). A band is present at 2.7 cm and has greater enzyme activity than a slower moving band at 2.3 cm.

**B Genome Comparison**

**Esterase Activity.** One isozyme pattern is produced by *G. anomalum* and *G. triphyllum* of the B genome (Figure 6). Bands 7.5 cm, 8 cm, and 8.5 cm are present in both species. *Gossypium anomalum* contains a minor band at 8.9 cm not present in *G. triphyllum*, and the latter has a minor band at 7 cm not found in the former.

**Leucine Aminopeptidase Activity.** The species of the B genome contain two major bands of similar mobility at 5.2 cm and 6.2 cm (Figure 7). The major band at 6.2 cm in *G. triphyllum* has much more activity than that of *G. anomalum*.

**Catalase Activity.** Figure 8 shows that each species has a minor band at 2.3 cm and a major band at 2.7 cm. The major band in *G. triphyllum* shows an increased amount of enzyme activity over that present in *G. anomalum*.

**C Genome Comparison**

**Esterase Activity.** Figure 9 shows that the species of the C genome contain a number of different isozyme patterns. The two varieties of *G. sturtianum* (varieties sturtianum and nandewarense) and *G. robinsonii* have similar zymograms. This similarity includes a minor
band at 7.5 cm and major bands at 8.5 cm and 8.9 cm. In addition, the
two varieties of *G. sturtianum* have major bands at 8 cm, whereas *G. robinsonii* has a minor band at this position. The low frequency zymogram
of variety nandewarense lack the minor band 7.5 cm but contain a minor
band at 8 cm similar to *G. robinsonii*.

An increase in band complexity and variability is observed within
*G. australis* and *G. bickii*. Minor band 7.5 cm and major bands 8 cm and
8.5 cm present in *G. sturtianum* and *G. robinsonii* are similarly observed
in these two species. However, quantitative differences in these bands
are observed. The zymogram of highest frequency within *G. australis*
contains a major band at 8.9 cm. Band 6.3 cm not present in the former two
species is observed in the latter two. Two zymograms of *G. australis*
contain a minor band at 7 cm while *G. bickii* has a large major band in
region 7-7.5 cm. The zymogram pattern of lowest frequency in *G. bickii*
contains a large major band in region 8.5-9 cm that is not present in
any of the other species within this genome. This 8.5-9 cm band may be
a combination of bands 8.5 cm and 8.9 cm present in the other species of
the C genome.

**Leucine Aminopeptidase Activity.** *Gossypium sturtianum* and *G.
robinsonii* produce similar zymograms (Figure 7). Major bands at 6.2 cm
and 5.7 cm are present in the former two species and in *G. australis* and
*G. bickii* but differ quantitatively. *Gossypium australis* and *G. bickii*
contain a major band at 5.2 cm that is not present in the other two spe-
cies of the C genome. In addition, *G. bickii* has a major band at 6.7
cm.
Catalase Activity. The species of the C genome contain a major band at 2.7 cm. *Gossypium australe* and *G. bickii* have an additional minor band at 2.3 cm (Figure 8).

D Genome Comparison

Esterase Activity. The species of the D genome contain a large amount of esterase variation (Figure 10). This variation is present within and between the species. For example, *G. aridum*, *G. harknessii*, *G. armourianum*, and *G. raimondii* show three different zymograms each, and *G. gossypioides* and *G. lobatum* have two and four different zymograms, respectively. Six different gel patterns are observed for *G. thurberi*. This latter observation, however, is based on the analysis of a large sample of seed from four different populations as was discussed previously. This result suggests that other zymograms might have been observed for other species if larger seed samples had been investigated.

*Gossypium klotzschianum* and *G. davidsonii* each have one isozyme pattern which is similar to each other. These zymograms contain major bands at 8 cm and 8.6 cm. Other closely related species such as *G. klotzschianum* and *G. davidsonii* show similar patterns. *Gossypium aridum* and *G. lobatum* considered to be in this category each produce one zymogram in high frequency comparable to one another. These gels have enzyme activity located at 8.3 cm, 8.7 cm, and 9.2 cm. The zymograms of those species having the next highest frequencies are similar except for some quantitative differences. These latter gels have bands 8.3 cm, 8.7 cm, and 9.2 cm plus an additional minor band at 7.6 cm.
Further comparison of esterase polymorphism within species of the D genome show that certain zymograms are present at a higher frequency in some species, while lower in others, and vice versa. For example, zymograms containing only two areas of enzyme activity, major bands 8.3 cm and 8.7 cm, are present at a low frequency in *G. aridum*, *G. lobatum*, and *G. harknessii*, while on the other hand, *G. gossypioides* produces this isozyme pattern in highest frequency and with a greater amount of enzyme activity. It can also be noted that the zymogram pattern of low frequency in *G. gossypioides* and *G. armourianum* contains major bands 8.3 cm and 8.7 cm plus a major isozyme at 7.6 cm, while *G. harknessii* has the same banding pattern at a higher frequency. Furthermore, a zymogram with the latter three isozymes plus a major band at 9.2 cm is present at a higher frequency in *G. armourianum* but at a lower frequency in *G. harknessii*. Quantitative differences between specific bands of these zymograms just compared can also be seen.

The high frequency zymogram of *G. thurberi* contains a band at 8 cm similarly present in *G. klotzschianum* and *G. davidsonii* but which exhibits greater enzyme activity than that of the latter species. Bands 6.5 cm and 7.3 cm may be unique to *G. thurberi*. The zymogram of lowest frequency in this species has a major band at 6.9 cm which partially corresponds in mobility to a major band of greater activity (band 7 cm) in *G. raimondii*. Except for this latter region, *G. raimondii* produces three different zymograms unique only to this D genome species. A major band is present in all three zymograms at 6.3 cm. Two of the gel patterns have a minor band at 5.8 cm and one has a minor band at 5.2 cm.
Leucine Aminopeptidase Activity. A greater amount of variation is observed between the species of the D genome than between those of the other genome groups (Figure 7). *Gossypium aridum* and *G. lobatun* contain a major band at 4.6 cm. However, the band in *G. aridum* produces more enzyme activity than that of the other species of the D genome. This 4.6 cm region of enzyme activity in the gel of *G. aridum* encompasses an area of 4.3-4.7 cm. This same region consists of two major bands, 4.3 cm in *G. klotzschianum* and *G. davidsonii*, and 4.6 cm in *G. lobatum*, *G. harknessii*, *G. armourianum*, *G. thurberi*, and *G. raimondii*. It is possible that this large region of activity in *G. aridum* consists of a combination of both bands 4.3 cm and 4.6 cm.

*Gossypium klotzschianum* and *G. davidsonii* produce similar banding patterns with major bands at 4.3 cm and 5.7 cm. The gels of *G. thurberi* and *G. raimondii* both displayed major bands at 4.6 cm and 5.2 cm. Band 4.6 cm is also observed in *G. harknessii* and *G. armourianum*. Band 5.2 cm is present as a minor band in *G. harknessii*, as a major band in *G. gossypioides* and as two bands in *G. armourianum* (band mobilities of 5 cm and 5.3 cm). *Gossypium harknessii* and *G. gossypioides* have band 5.7 cm similar to *G. klotzschianum* and *G. davidsonii*.

Catalase Activity. Comparison of the catalase activity of the species of the D genome to all other genome groups shows that there is a greater amount of enzyme activity in the former species group than in the latter (Figure 8). Major bands at 2.6-3.4 cm, 2.3 cm, and 1.7 cm are present in all of the D genome species. Band 2.3 cm is a minor band in *G. thurberi*. *Gossypium harknessii*, *G. klotzschianum*, *G. davidsonii*,
and *G. gossypioides* have a minor band at 0.7 cm and the latter three species have enzyme activity at 1.3 cm.

**E Genome Comparison**

**Esterase Activity.** Three species of the E genome, *G. stocksii*, *G. somalense*, and *G. areysianum* show a number of different zymograms (Figure 9). The gel pattern of *G. incanum* is similar to the highest frequency gel of *G. areysianum*. Major bands are located at 7.3-7.6 cm, 7.8 cm, and 8.5 cm, and a minor band is present at 6.3 cm. The low frequency gel of *G. areysianum* with two major bands, 7.3-7.6 cm and 7.8 cm, is identical to the zymogram of highest frequency in *G. stocksii*. This observation is similar to the relationship of the zymogram frequencies observed in the D genome.

The overall isozyme patterns of *G. stocksii* and *G. somalense* differ from each other. However, some major and minor bands have similar mobilities within these two species. These major and minor bands also compare closely in mobility to those found in the other species of the E genome. For example, bands 7.3-7.6 cm and 7.8 cm are found in zymograms of these two E genome species as well as *G. areysianum* and *G. incanum*. Band 8.5 cm is present in at least one gel of each species and band 6.3 cm is present in *G. somalense*, *G. areysianum*, and *G. incanum*.

*Gossypium somalense* has a minor band at 7 cm which is present as a major band in the gel of the lowest frequency of *G. stocksii*. This low frequency gel of *G. stocksii* also contains a band at 8.9 cm that is not present in any of the other species of the E genome. The gel pattern of highest frequency in *G. somalense* compares more closely to that
of *G. areysianum* and *G. incanum*. In *G. somalense* a zymogram containing one band of high enzyme activity in region 7.3-7.8 cm may be comparable to the bands of *G. stocksii* present in this same region. The former enzymes, however, display more activity.

**Leucine Aminopeptidase Activity.** *Gossypium stocksii* contains one band with a large amount of enzyme activity in region 5-5.5 cm (Figure 7). The other three species of the E genome have two bands of similar activity at 5.2 cm and 5.7 cm.

**Catalase Activity.** A major band at 2.7 cm is present in all four species of the E genome. This 2.7 cm band of *G. incanum* contains more enzyme activity than that observed in the other three species. A minor band is present at 2.3 cm in *G. somalense* and *G. areysianum*. This 2.3 cm band is observed as a major band in *G. incanum* (Figure 8).

**F Genome Comparison**

**Esterase Activity.** *Gossypium longicalyx* is the only species known in this genome group (Figure 9). Three distinct major bands, 7.3 cm, 8.5 cm, and 9.5 cm, and one minor band at 8 cm are observed.

**Leucine Aminopeptidase Activity.** Two major bands at 5.2 cm and 6.2 cm distinguishes this species from the other genome groups (Figure 7).

**Catalase Activity.** One large band of major enzyme activity at 2.7 cm is present in this F genome species (Figure 8).
Comparison of the Enzyme Banding Patterns Between the Six Genomes

Esterase Activity

Examination of Figures 6, 9, 10, and 11 shows that G. herbaceum and G. arboreum of the A genome are the only species in the genus Gossypium which produce esterase isozymes with low activity. The other genomes have species showing all major band activity or combinations of major and minor bands. Thus quantitative as well as qualitative differences play a role in distinguishing between genome groups.

Esterase isozymes are present which have similar mobilities within the six genomes. For example, the A genome varieties have more bands than the species of the B genome. However, bands 7 cm, 7.5 cm, and 8.9 cm are found in both groups. Bands with similar mobilities to these are also present in some species of the C, D, and E genomes. However, quantitative variations in these latter three bands distinguishes the species of the different genome groups. The species of the B genome have bands at 8 cm and 8.5 cm which are not present in the A genome species but are observed in most of the species of the C and E genomes. In contrast, the species of the A genome contain enzyme activity at 6.3 cm, not present in the B genome, but which is present in certain species of the C, D, and E genomes.

Gossypium longicalyx of the F genome contains an overall banding pattern unique to itself with four bands separated distinctly from one another. A major band is noted in region 9.5 cm which is not observed within any of the other genomes. However, this 9.5 cm band is present as a minor band in the species of the natural allotetraploids. The
Figure 11. Polyacrylamide gel electrophoretic esterase spectra of the zymograms occurring in highest frequencies in the diploid and allotetraploid species of Gossypium.
other two major bands of *G. longicalyx* have mobility rates 8.5 cm and 7.3 cm. Band 8.5 cm is present in the B, C, and E genomes and band 7.3 cm is observed in *G. thurberi* of the D genome. *Gossypium longicalyx* has a minor band at 8 cm which is present as a major band in *G. thurberi*, *G. davidsonii*, and *G. gossypioides* of the D genome. This 8 cm band, with quantitative differences between species, is also observed in the B and C genomes.

The species of the D genome exhibit a greater diversity of esterase banding patterns (e.g., *G. raimondii*, region 5.2 cm to 7 cm, to *G. thurberi*, region 6.5 cm to 9.2 cm) than those of species within the other genomes. Some similarities, however, can be noted between the D genome species and the other genomes. For example, the bands at 6.3 cm and approximately 7 cm of *G. raimondii* are present in the A genome varieties. *Gossypium thurberi*, *G. davidsonii*, and *G. klotzschianum* have major bands at 8.6 cm and 8 cm which are observed in zymograms of species in the A and B, and C and F genomes, respectively.

Of interest is the wider range of variability in enzyme activity within the zymograms of the genomes that are not located in Africa (Figure 11). This continent was theorized to be the center of origin for the genus *Gossypium* by Hutchinson, Silow, and Stephens (1947) and contains genomes A, B, and one species of E and F (Figure 1). Species not included on this continent (genomes C, D, and three species of E) show a great deal more variability with respect to their banding patterns than those of the former four groups.
Leucine Aminopeptidase Activity

Compared to the esterase zymograms, less leucine aminopeptidase isozyme variability is observed between species of each genome group (Figure 7). The species of each genome have an overall banding pattern distinct from the other genomes. Band 5.2 cm of the varieties in the A genome is present in both species of the B genome, two species (G. australis and G. bickii) of the C genome, all species of the E and F genomes and five species of the D genome (G. harknessii, G. armourianum, G. thurberi, G. raimondii, and G. gossypioides). Quantitative variation in band 5.2 cm is present in most of the genomes. Similarly, band 5.7 cm is present in a number of species from genomes A, C, D, and E. The species of genomes B, C, and F contain band 6.2 cm. Band 4.6 cm, present in the species of the D genome, and band 6.7 cm of G. bickii (C genome), are not observed within any other genome.

Catalase Activity

A similar situation can be summarized for the catalase isozyme patterns as was stated for leucine aminopeptidase activity. Except for the increase in the number of bands in the species of the D genome, little variation in isozyme patterns is observed within and between species of the different genomes (Figure 8). Bands 2.3 cm and 2.7 cm are present in most of the species analyzed. However, the latter two bands are distinguished within each genome by quantitative differences. Of interest is the observation that the D genome species have much more catalase activity than the other genomes. The species of the D genome range from three to five distinct bands whereas those of the other genomes contain one to two bands.
Analysis of the Natural Allotetraploid Species and Varieties

Esterase Activity

One zymogram pattern is observed for *G. tomentosum* and for the varieties of *G. barbadense* (Pima S x P, Pima S-2, Menoufi and Sea Island) examined (Figure 1). The varieties of *G. hirsutum* show three different types of gel patterns. These three patterns are represented by Rowden, Stoneville 7A, and Kechi. Varieties having banding patterns similar to Rowden include Acala 44-10-1, Acala 1517A, Paymaster 54B, CB3051, and Lockett 4789; those like Kechi include Mexican Big Boll, Hibred, Macha, Clevewilt, King 82, and Contextum; and one variety, Hopi Sacaton, has a banding pattern similar to Stoneville 7A.

*Gossypium tomentosum* and the three varieties of *G. hirsutum* are alike in exhibiting a major band at 5.8 cm and 6.3 cm. *Gossypium tomentosum* has a major band at 7 cm which is also present in Rowden. This latter band is observed as a minor band in Stoneville 7A. *Gossypium barbadense* has a minor band in region 5.8 cm and major bands in regions 6.3 cm and 7 cm. However, these latter two bands show more enzyme activity than that observed in *G. tomentosum* and *G. hirsutum*. A minor band is present at 7.7 cm, 8.9 cm, and 9.5 cm in the three allotetraploid species. Band 8.9 cm is absent in Kechi of *G. hirsutum* and the enzyme activity at 7.7 cm of Rowden is greater than that of the other species and varieties.

Leucine Aminopeptidase Activity

Figure 7 shows that *G. tomentosum* and *G. hirsutum* contain three major bands at 4.6 cm, 5.2 cm, and 5.7 cm. *Gossypium barbadense*
similarly contains these bands but with an increase in enzyme activity. In addition this latter species has a major band at 4.2 cm that is not present in the former two species.

**Catalase Activity**

Three bands of catalase activity are located in the natural allotetraploids (Figure 8). The bands are at 1.7 cm, 2.3 cm, and 2.6-3.3 cm. Band 1.7 cm is a minor band in *G. tomentosum*. The catalase activity of bands 1.7 cm and 2.6-3.3 cm is much greater in *G. barbadense* than that observed in the other two natural allotetraploids.

**Comparison of the Three Natural Allotetraploids to Two Varieties of the A Genome and One Species of the D Genome and Their Synthetic Mixtures**

**Esterase Activity**

When the extracted proteins of *G. herbaceum* var. africanum (G304), *G. hirsutum* var. africanum (G25) and *G. raimondii* (D5) are combined into a synthetic mixture, a gel containing all of the bands of both genomes is produced (Figure 12). This observation is also true for variety G25 (A2) and *G. raimondii*. The individual gels of G304 and *G. raimondii* and their synthetic mixture can be compared to the esterase banding patterns of the allotetraploids. Except for some quantitative enzyme differences, *G. tomentosum* and the varieties of *G. hirsutum* with similar banding patterns to Rowden and Stoneville 7A compare closely to the synthetic mixture of the A1 + D5 mixture. Although *G. barbadense* is similar to the synthetic mixture in regard to position of esterase bands, quantitative enzyme differences of bands 5.8 cm, 6.3 cm, and 7 cm distinguish *G. barbadense*. The band
Figure 12. Polyacrylamide gel electrophoretic esterase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids.

Upper spectra: Comparison between *G. herbaceum* var. *africanum* (G304), *G. raimondii* (Dgenome), their synthetic mixture, and the three natural allotetraploids: *G. tomentosum*, *G. barbadense*, and *G. hirsutum*.

Middle spectra: Comparison between *G. arboreum* var. *G25* (A genome), *G. raimondii* (Dgenome), their synthetic mixture, and the three natural allotetraploids: *G. tomentosum*, *G. barbadense*, and *G. hirsutum*.

Lower spectra: Comparison between *G. arboreum* var. *G24* (A genome), *G. thurberi* (Dgenome), their synthetic mixture, and their synthetic allotetraploid: 2(A,D), AZ239.
Figure 12. Polyacrylamide gel electrophoretic esterase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids.
located at 9.5 cm, in the three allotetraploids, seems to be absent in the varieties of the A genomes, D genome, and the synthetic mixture.

A similar discussion for the $A_1 + D_5$ mixture can also apply to the $A_2 + D_5$ combination when compared to the natural allotetraploids. However, minor bands 7.5 cm and 8.6 cm, present in variety G25 and the synthetic mixture, are absent in the allotetraploids.

**Leucine Aminopeptidase Activity**

Band 5.2 cm is present as a single major component in the natural allotetraploids. In the synthetic mixtures, this 5.2 cm band contains two regions of enzyme activity at 5.2 cm and 5.3 cm and is due to a slight difference in mobility of the respective bands in the $A_1$ and $D_5$ parents. Apart from this minor difference, the synthetic mixture $A_1 + D_5$ and $A_2 + D_5$ are identical to *G. tomentosum* and *G. hirsutum*. *Gossypium barbadense* also has bands of similar mobility to *G. tomentosum* and *G. hirsutum*. The bands of *G. barbadense*, however, contain more isozyme activity than that of the latter two species. In addition, this latter species has a major band at 4.2 cm that is not present in the other natural allotetraploids and the $A_1$ and $D_5$ species (Figure 13).

**Catalase Activity**

Both mixtures of $A_1 + D_5$ and $A_2 + D_5$ have identical banding combinations. Bands 2.6-3.3 cm, 2.3 cm, and 1.7 cm of *G. tomentosum* and *G. hirsutum* compare closely to the synthetic mixtures. *Gossypium barbadense* has greater enzyme activity in bands 1.7 cm and 2.6-3.3 cm than that of the other two species (Figure 14).
Figure 13. Starch gel electrophoretic leucine aminopeptidase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids.

Upper spectra: Comparison between G. herbaceum var. africanum (G304), A₁ genome, G. raimondii (D₅ genome), their synthetic mixture, and the three natural allotetraploids: G. tomentosum, G. barbadense, and G. hirsutum.

Middle spectra: Comparison between G. arboreum var. G25 (A₂ genome), G. raimondii (D₅ genome), their synthetic mixture, and the three natural allotetraploids: G. tomentosum, G. barbadense, and G. hirsutum.

Lower spectra: Comparison between G. arboreum var. G24 (A₂ genome), G. thurberi (D₁ genome), their synthetic mixture, and the synthetic allotetraploid: 2(A₂D₁), AZ239
Figure 13. Starch gel electrophoretic leucine aminopeptidase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids.
Figure 14. Starch gel electrophoretic catalase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids.

Upper spectra: Comparison between *G. herbaceum* var. africanum (G304), *A1* genome, *G. raimondii* (D5 genome), their synthetic mixture, and the three natural allotetraploids: *G. tomentosum*, *G. barbadense*, and *G. hirsutum*.

Middle spectra: Comparison between *G. arboreum* var. G25 (A2 genome), *G. raimondii* (D5 genome), their synthetic mixture, and the three natural allotetraploids: *G. tomentosum*, *G. barbadense*, and *G. hirsutum*.

Lower spectra: Comparison between *G. arboreum* var. G24 (A2 genome), *G. thurberi* (D1 genome), their synthetic mixture, and the synthetic allotetraploid: 2(A2D1), AZ239.
Figure 14. Starch gel electrophoretic catalase spectra of a comparison between the possible parentals, their synthetic mixtures, and the allotetraploids.
DISCUSSION

Sheen (1970), as a result of an extensive investigation of peroxidase isozymes in the genus *Nicotiana*, considered the following arguments in his interpretation of the zymograms:

1. An increase of the molecular heterogeneity observed within the enzymes is likely to amplify the degree of divergence rather than similarity among species. This molecular heterogeneity can result from a number of factors, for example, (a) proteins which differ in primary structure and which are probably produced by multiple gene loci, (b) proteins with identical primary structures but which have undergone secondary alterations, and (c) enzymes in a heterozygous state due to point mutations.

2. Isozyme bands of similar mobility in the same or different species reflect similar base sequences in structural genes which contribute polypeptide subunits to a migrating entity. Without the genetic studies that accompany the differences in mobility of isozyme patterns, it is difficult to derive an exact genetic relationship of these bands between the species studied.

3. If the gene loci of specific enzymes studied in *Gossypium* species are highly polymorphic, comparisons between inbreeding and small populations could tend to exaggerate the differences between them; i.e., polymorphism tends to bias downward the estimates of the genetic identity of isozyme genes among species. In addition to this argument
considered by Sheen (1970), Shaw (1965) has stated that the data derived from small samples depend on a number of factors. These factors include: (a) the size of the original colony samples, (b) the number of generations inbred before analysis, and (c) the selective factors involved in the experimental environment. However, as long as the enzymes under study have not been selected for or against in the laboratory populations, much can be added to the general picture of the variability in frequency of electrophoretic mutants. Also, Shaw (1965) has considered that the occurrence of low frequency variable isozyme patterns are probably due to chance mutations. Those of a higher frequency probably involve something more than mutation and natural selection and may include genetic drift, variable mutation rates for different genetic sites, and close linkage with genes which have selective values. This author states further that some enzymes which show little or no variation within a particular species may have either achieved "perfection" at a particular point in time in the course of evolution, or cannot tolerate change.  

4. Since various concentrations of protein materials were used to determine the presence or absence of enzyme activity, missing bands may also reflect a change in the nucleotide sequence of a structural or regulatory gene.

Esterase Isozyme Polymorphism in Natural Populations of Gossypium Thurberi

Except for differences in leaf petal pigmentation and boll size, Walter E. Bryan (Department of Plant Breeding, The University of Arizona, personal communication, 1970) has observed very little morphological
variation within and between populations of *G. thurberi* throughout southern Arizona and Sonora, Mexico. The presence of six types of esterase zymograms for *G. thurberi*, occurring at varying frequencies within and between the four locations studied indicates that notable genetic variability does exist at the molecular level (Figure 5, Table 4).

To account for the origin of these isozyme patterns (since genetic studies have not been conducted) several distinct possibilities should be considered: (a) the presence of a variety of different combinations of alleles; (b) the presence of nonallelic gene combinations which code for multimeric enzymes; (c) a number of different genes which code for functionally related monomeric enzymes; and (d) a combination of some or all of the above possibilities. As to which of these alternatives is most likely, it can be noted from the data that the 8 cm band is the only one present in all of the zymograms of *G. thurberi* (Figure 5). Thus, all of these isozyme patterns may have arisen from this main esterase through mutation within a single gene (intragenic variation) or through duplication of a single gene, which then underwent mutation (intergenic variation). Hybridization studies of isogenic lines which produce each of these esterase zymograms, however, are needed before anything of a definite nature can be postulated.

Scogin (1969) demonstrated the existence of a genetically based polymorphism with respect to isozyme patterns within natural populations of *Baptisia* (Leguminosae). Therefore, to predict a possible taxonomic or physiological relationship between species with regard to a given
isozyme pattern, intraspecific variation should be evaluated. The results with the esterases in _G. thurberi_ similarly suggest that intraspecific variation should be evaluated before interspecific analyses are undertaken.

**Analysis of the Genome Groups of the Genus Gossypium**

The polyacrylamide and starch gel electrophoresis of dormant seed esterase, and leucine aminopeptidase and catalase, respectively, show that the species and/or varieties observed within each genome have banding patterns of enzyme activity that are more similar to one another than to members in other genome groups (Figures 6-11). These results support the classification of the diploid species into the presently recognized six genome groups (A-F). The following discussion includes a comparison of the classical data to that derived from the biochemical analyses.

The two species of the A genome, _G. herbaceum_ (A₁) and _G. arboreum_ (A₂), have long histories of cultivation in Africa and Asia. The many varieties of these species are considered as true cottons (Hutchinson 1954). Hutchinson has presented data indicating that _G. herbaceum_ variety africanum is the only true wild type within the A genome.

Gerstel (1953), in the analysis of metaphase I chromosomes of hybrids of _G. herbaceum x G. arboreum_, observed eleven bivalents and one figure of four chromosomes (reciprocal translocation). Hybrids between _G. arboreum x G. anomalum_ (B₁ genome, wild african species) also exhibited a figure of four chromosomes. The cross between _G. herbaceum x_
G. anomalum produced no multivalent figures. Since the genome make-up of G. herbaceum is similar to the wild B genome species, it was suggested that G. arboreum arose from G. herbaceum by the reciprocal translocation. In addition, Gerstel (1953) and Phillips (1963), through the production of fertile hybrids from crosses between the species of the A and B genomes, suggested that the A genome was more closely related to the B than to any of the other genomes. It was further suggested that one genome may have evolved from the other.

Comparison of esterase banding patterns between the varieties of the two A genome species shows that the same group of isozymes is present. The differences between varieties are based on the presence or absence of certain bands. Overall, a small increase in banding complexity is observed in the varieties of G. arboreum when compared to the varieties of G. herbaceum. Hutchinson (1959) has stated that G. arboreum evolved from G. herbaceum under cultivation since no wild varieties of the former are known. This slight increase in complexity of the cultivated varieties could come about through selection by man for greater modification of those characters which are important agronomically. No variations in the banding patterns of leucine aminopeptidase and catalase are observed between the varieties of the two A genome species.

The presence of the translocation within the A2 genome evidently had little effect on the expression of these three enzyme systems since no major differences in isozyme patterns distinguish either the A1 or the A2 genomes.
Comparison of the esterase, catalase, and leucine aminopeptidase isozyme patterns between the species of the A and B genomes shows both quantitative and qualitative differences. Similarly, but to a lesser extent, these differences distinguish the two species of the B genome for esterase activity. However, only quantitative differences distinguish the leucine aminopeptidase and catalase zymograms of the B genome species. Generally, fewer differences in isozyme patterns of the three enzyme systems are present between the species within the A and B groups than between the A and B genomes. These results give further support to the taxonomic classification of these two genomes.

Fryxell (1965b) re-evaluated the C genome of Australia. From morphological, geographical, and cytological analyses, G. robinsonii and G. sturtianum were placed together under the section Sturtia of the subgenus Sturtia. Except for the absence of a minor esterase band in the lower frequency gel of G. sturtianum var. nandewarense, only a quantitative difference distinguishes the varieties of G. sturtianum from G. robinsonii. No differences are observed in the leucine aminopeptidase and catalase activity of these two species.

Gossypium bickii and G. australe were observed to be more closely related to each other than to the other species of the C genome and subsequently were placed in subsection Hibiscoidea of the section Hibiscoidea (Fryxell 1965b). Both G. australe and G. bickii produce a number of esterase bands present in G. sturtianum and G. robinsonii. However, the former two species form closely related esterase zymograms which are
distinct from the latter two species. Similar observations are noted for the leucine aminopeptidase and catalase zymograms.

Brown and Menzel (1952a) studied polygenic hybrids made between different species of the genome groups in *Gossypium*. They observed that there was nearly as much chromosomal homology between $B_1$ and $C_1$ as between the $A$ and $B$ or the $A$ and $C$ genomes. Comparison of the zymograms of esterase, leucine aminopeptidase, and catalase shows that the species of the $C$ genome are made up of a combination of both $A$ and $B$ genome enzymes. These enzyme studies further indicate that the species of the $A$, $B$, and $C$ genomes contain a number of homologous genes as indicated by the chromosomal homology of the hybrids.

Saunders (1961) summarized the relationship of the species within the $E$ genome. Cytological data had shown that the hybrid *G. somalense* × *G. areysianum* produced a very high homology between the two chromosome sets. Similarly, this homology was also found between the chromosomes of *G. stocksii* and the above two species. Morphologically, *G. incanum* is closely related to *G. areysianum* and *G. somalense*. Geographically, however, *G. stocksii* and *G. somalense* are positioned at the extremes of the locality in which the $E$ genome species have been found (Figure 1); i.e., *G. stocksii* is located in India and southeast Arabia, and *G. somalense* from Somaliland to Kenya of Africa.

In all three enzyme systems the overall banding patterns of *G. stocksii* and *G. somalense* do not match closely. However, these species do have a number of bands in common showing their genome relationship. These observations seem to coincide with the geographical rather than
the cytological data. *Gossypium areysianum* and *G. incanum* are both located in the same region of southern Saudi Arabia; this region isolates *G. stocksii* from *G. somalense*. The banding patterns of *G. areysianum* and *G. incanum* coincide more closely to each other than to the other species of the E genomes. *Gossypium areysianum*, *G. incanum*, and *G. somalense* in turn are more closely related in their banding patterns than any of these three species are to *G. stocksii*. This observation is in agreement with the morphological data. Overall, the isozyme patterns of the E genome are unique when compared to those of the A, B, and C genomes. This latter observation further supports the cytological work of Douwes (1953) and Brown and Menzel (1952b) in which the individuality of this genome was suggested.

Phillips and Strickland (1966) made cytological and hybridization analyses of a cross between *G. hirsutum* x *G. longicalyx*. The analyses of this triploid hybrid indicated that *G. longicalyx*, originally thought to be a species of the E genome, should be given a new classification which they designated as F. The distinct banding pattern of *G. longicalyx*, i.e., the presence of major esterase activity at 9.5 cm, and the leucine aminopeptidase major band at 6.2 cm, not present in any species of the E genome, lends support to the individuality of this F genome species. Several bands of esterase, leucine aminopeptidase, and catalase activity in *G. longicalyx*, however, have migration rates similar to those present within the E genome. This observation indicates that the species of the E and F genome are related. Based on analyses of chromosome size and bivalent formation in a triploid hybrid, Phillips
and Strickland (1966) pointed out that *G. longicalyx* was more closely related to the A genome rather than the E genome. No such conclusions can be drawn from the enzyme data presented here.

Incompatibility due to genetic causes rather than to structural changes of chromosomes has limited interspecific hybridization analyses between some species of the D genome (Menzel and Brown 1955). Analysis of the metaphase pairing of chromosomes in hybrids between compatible D genome species showed that the mean number of bivalents formed was approximately twelve. In most cases, however, the hybrids formed from these crosses were sterile, but the high number of bivalents was an indication of the genetic relatedness of the D genome species. Saunders (1961) pointed out that the variability and the genetic differences within the D genome are related to the fact that the species are geographically isolated and no longer vigorously colonizing. Electrophoretic analysis allows one to circumvent these problems of genetic incompatibility and hybrid sterility in determining species relationships. The relationship of all species can be determined directly by comparing their protein and/or enzyme banding patterns.

Much variability of the esterases is observed within and between the species of the D genome. In a number of cases, specific isozyme patterns occurred with a low frequency in one species and a high frequency in another, whereas the reverse situation could be obtained with other isozyme patterns. For example, in *G. harknessii* a zymogram with three esterase bands occurs with a high frequency of 0.667. Within this species a zymogram with four esterase bands occurs in a lower frequency...
of 0.209. In *G. armourianum*, the former zymogram has a lower frequency of 0.250 and the latter is in highest frequency with 0.500. Also noted is that *G. aridum*, *G. lobatum* and *G. harknessii* each contain a zymogram in low frequency with two major esterase bands. This zymogram pattern is present in high frequency in *G. gossypioides* with an increase in enzyme activity. A similar condition is noted with *G. stocksii* and *G. areysianum* of the E genome. These data indicate that possibly different combinations of alleles coding for the esterases are present within the species of these genomes. The varying zymogram patterns may be a reflection of the selective pressures operating to produce the most stable genetic complement for each species in its natural habitat. Also, it cannot be discounted that these isozyme patterns may be due to a differential genetic expression of the alleles in the organism, or due to the ease of extraction of the different isozymes from these species.

Endrizzi (1957) showed cytologically that *G. lobatum* and *G. aridum* were closely related. *Gossypium harknessii* was shown to cross freely with *G. armourianum* (Kearney 1957). The highest chiasma frequency among the D genome species was produced in crosses between *G. klotzschianum* and *G. davidsonii* (Phillips 1966). These comparisons are further supported by the isozyme patterns of the three enzyme systems; the more closely related the species are genetically, the more similar are their banding patterns. Hutchinson, Silow, and Stephens (1947) indicated that *G. raimondii* was closely related to *G. thurberi*. This latter observation is supported by the leucine aminopeptidase activity and in part by the catalase isozyme patterns. However, the zymograms
of esterase do not support this relationship. *Gossypium raimondii* produces esterase isozymes unique to itself.

Cherry, Katterman, and Endrizzi (1970), using the technique of polyacrylamide gel electrophoresis, investigated the proteins from dormant seeds of species and varieties within the six genome groups of the genus *Gossypium*. Similar conclusions as to the species relationships within and between these six genomes were developed from the protein data as that observed for the enzyme systems in this present study.

**The Relationship Between Species in Africa to the Species Removed From This Postulated Center of Origin**

Hutchinson, Silow, and Stephens (1947) stated that the center of origin of Asiatic species was probably the Lower Indus Valley. The ancestor of these species probably arose in the southern periphery of South Africa. Since species belonging to four different genome groups (A, B, E, and F) are located on the continent of Africa, the authors felt that this center of diversity was the origin of the genus *Gossypium*. From this region one species group developed in Australia (C genome) and one in America (D genome). Two of the genome groups of Africa (A and E) have spread into Asia. Brown and Menzel (1952a), upon examination of polygenic hybrids made between different species of the genomes, indicated that there was nearly as much chromosomal homology between B1 and C1 species as between the species of the A and B or the A and C genomes. Hybridization of D genome species with species from other genome groups has been difficult (Saunders 1961). *Gossypium longicalyx* (F genome) was
shown to cross with \textit{G. incanum} but not with any of the other E genome species (Phillips and Strickland 1966).

Vavilov (1926) postulated that the center of origin of species is indicated by the position of greatest heterogeneity. As populations become isolated from the center of origin, additional genetic diversity can lead to increasing differentiation of the genetic make-up of the species (Hutchinson, Silow, and Stephens 1947). Eventually the isolated populations will diverge to a point of inability to hybridize.

From this summary of the relationship between the species of the different genome groups, it is of interest to determine whether one can derive a similar relationship using the isozyme patterns as a criterium.

The species of the genomes (i.e., C, D, E, and F), removed from the postulated center of origin (South Africa) of the genus \textit{Gossypium}, tend to show an increase in the amount of major band activity over that produced by the suggested ancestor species (A and B genomes). Quantitative differences between species within and between genomes may reflect significant differences in the dose of specific genes responsible for enzyme formation or may involve major differences in control mechanisms that regulate the expression of the structural genes. These factors may play a role in differentiating the species as they move into new environmental habitats away from the center of origin.

Although a number of esterase and leucine aminopeptidase bands coincide in mobility between the genome groups, each genome expresses its own overall isozyme patterns. Examination of these two enzyme systems shows that the C, E, and F genomes are made up of combinations of
band mobilities from both the A and B genomes plus some additional bands. Quantitative differences between bands of the different genome groups are observed. An increase in isozyme diversity is present within and between the species of the D genome over that of the other genomes of *Gossypium*. In addition, a qualitative and quantitative increase in catalase activity further distinguishes this D genome. Less catalase isozyme variability is present between the other genome groups than that observed for esterase and leucine aminopeptidase. Similar to the enzyme data, the studies with seed proteins (Cherry, Katterman, and Endrizzi 1970) showed that those genomes located away from the postulated center of origin increased in complexity and variability of banding patterns.

These data, using proteins and enzymes, may possibly indicate that the basic genetic make-up of the species located within the postulated center of origin (South Africa) is present within the species of the genome groups removed from this area. However, the additional isozyme complexity of the latter species may have evolved through mutations and the differential selection of these genes.

**Analysis of the Allopolyploids of the Genus *Gossypium***

The genus *Gossypium* contains two natural (now extensively cultivated) allotetraploids, *G. barbadense* and *G. hirsutum*, with their centers of origin in South and Central America, respectively. *Gossypium tomentosum*, endemic to the Hawaiian Islands, is a wild allotetraploid. It is well known that the three allopolyploid species cross freely to give vigorous and fertile $F_1$ hybrids. However, an extensive genetic breakdown occurs in the $F_2$. 
From hybridization studies, Skovsted (1934) concluded that the new world allotetraploids probably originated from a cross between two diploid species, one of which was an Asiatic type (A genome) and the other a wild American species. Harland (1940) and Beasley (1940) produced synthetic allotetraploids between the diploid American species, G. thurberi, and the diploid Asiatic species, G. arboreum. These synthetic hybridization studies suggested that the allotetraploid species might be derived from a natural cross such as this.

The spontaneous occurrence of allopolyploidy in diploid species hybrids of Gossypium was shown by Brown (1951). Doubling of the chromosome number occurred spontaneously in a hybrid of G. davidsonii x G. anomalum. The resulting fertile allotetraploid progeny had the characteristics of both parent species.

Crosses of G. hirsutum with G. arboreum and G. herbaceum, made by Gerstel (1953) and Menzel and Brown (1954), produced hybrids with certain kinds of translocations. These cytological data led them to conclude that the A genome of G. hirsutum was more similar to the genome of G. herbaceum (A1) than to the genome of G. arboreum (A2). Further work with the allotetraploids by Gerstel and Sarvella (1956) revealed that hybrids involving G. herbaceum, G. arboreum, G. tomentosum, G. barbadense, and G. hirsutum contained the same types of chromosomal translocations. These data indicated a possible monophyletic origin for the three allotetraploid species.

As for the possible origin of the D genome of the allotetraploids, Hutchinson, Stephens, and Dodd (1945) studied the seed hair
characteristics of *Gossypium* and concluded that of all the known *D* genome species, only *G. raimondii* could combine with *A* genome species and produce an allotetraploid having spinnable seed hairs. It has been shown in cytological and genetic studies that of all the *D* species, *G. raimondii* is more closely related to the *D* genome of the allotetraploid new world cottons than any of the other *D* species (Sarvella 1958, Gerstel and Phillips 1958, and Phillips 1963, 1964).

Through the use of the classical techniques, genomes similar to *G. herbaceum* (*A*<sub>1</sub>) and *G. raimondii* (*D*<sub>5</sub>) were postulated to be the most likely ancestors of the natural allotetraploids of *Gossypium*. The use of biochemical techniques such as polyacrylamide and starch gel electrophoresis of enzymes can add a new dimension to this area of study. Studies (West and Garber 1967, and Vaughan and Waite 1967a,b) of enzyme zymograms of amphidiploids showed that they compared closely to the additive isozyme patterns of the parent species when the latter were combined in a synthetic mixture. These data indicated that such studies could be performed on the synthetic and natural allotetraploid species of cotton.

Seed esterase, leucine aminopeptidase, and catalase activities from a synthetic allotetraploid (AZ239), which originated in the 1930's at Raleigh, North Carolina, were examined by electrophoresis (Figures 12-14). This allotetraploid was formed from a cross between a variety of *G. arboreum* (*A*<sub>2</sub>) and *G. thurberi* (*D*<sub>1</sub>). A synthetic mixture of the seed extracts was formed from the parents (*G. arboreum*, var. G24, and *G. thurberi*) and their isozyme patterns were compared with that of the
allotetraploids. When the parent species were combined, an additive zymogram for each of the three enzyme systems was formed in the synthetic mixture. These additive zymograms compared closely to the isozyme patterns of AZ239.

Recently, Muramoto (1969) synthesized a triploid from a cross between *G. hirsutum* (var. Experimental Acala 44-10-1) and of *G. sturtianum* (var. sturtianum). The chromosomal number of the triploid was doubled (hexaploid, Experimental 6x-3) with the aid of colchicine. As observed with the synthetic allotetraploid (AZ239), the banding patterns of both the triploid and hexaploid seed esterases compared well with the additive isozyme patterns of the parents in the synthetic mixture (Figure 15).

It should be noted that the comparisons made above with the synthetic allopolyplploids were based on the gels occurring in highest frequency. The highest frequency gel showed a good additive relationship of the parent zymograms to the synthetic mixture and to the allopolyplploids. In addition, comparisons of synthetic mixtures of various species within and between genome groups showed that the synthetic allopolyplploids compared best to the synthetic mixtures of their respective parents (Figures 2-4 and 12-15). These observations can also be summarized for the natural allotetraploids (Figures 12-14). The possibility cannot be disregarded, however, that a plant containing the genetic make-up for one of the low frequency zymograms may have contributed to the formation of the original allopolyplploids.
Figure 15. Polyacrylamide gel electrophoretic esterase spectra of a comparison between G. hirsutum var. Acala 44-10-1, 2(AD)1, G. sturtianum var. sturtianum (C1), their synthetic mixture, and the synthetic triploid and colchicine-induced hexaploid, Experimental 6X-3.
The results from the comparisons made above indicate that similar analyses can also be made with the natural allotetraploids, *G. tomentosum*, *G. barbadense*, and *G. hirsutum* to possibly determine their parental origins. The analyses are discussed below.

The synthetic mixture made up of the *A₁* genome (G304) and the *D₅* genome (*G. raimondii*) shows an additive pattern of the enzyme activity present in the two diploids. This additive pattern is produced in all three of the enzyme systems (Figures 12-14). Except for large quantitative differences in specific esterase bands, *G. tomentosum*, *G. barbadense*, and several of the varieties of *G. hirsutum* compare closely to the synthetic mixture of the two alleged parents. However, qualitative differences do exist in some of the varieties of *G. hirsutum*. The comparisons with leucine aminopeptidase and catalase gave similar results as the esterase zymograms but with an additional leucine aminopeptidase band at 4.2 cm for *G. barbadense*.

The synthetic mixture of esterase activity involving the *A₂* genome (var. G25) and *D₅* genome (*G. raimondii*) differs slightly from that of the *A₁ + D₅* mixture (Figure 12). Minor bands 7.5 cm and 8.6 cm, present in variety G25 and the synthetic mixture are absent in the allotetraploids. Since no differences in leucine aminopeptidase and catalase activity were observed between *G. herbaceum* and *G. arboreum* (Figures 13 and 14), both species produced identical synthetic mixtures when combined with *G. raimondii*. Therefore, no conclusions as to which *A* genome contributed to the allotetraploids could be made.
The greater similarity of the esterase banding patterns of the allotetraploids to those of the *G. herbaceum*-*G. raimondii* mixture, however, provides some additional support for the hypothetical origin of the allotetraploids, i.e., ancestral forms whose genomes were similar to the genomes of *G. herbaceum* and *G. raimondii*.

It was shown that the esterase, leucine aminopeptidase, and catalase activity of the synthetic allopolyploids could be duplicated by mixing the enzyme extracts of their parents. Therefore, the synthetic mixtures of *A₁ + D₅* can indicate with some degree of approximation the type of banding patterns for seed enzymes that the original ancestral 2*(AD)*ₙ allotetraploid hybrid may have contained. Examination of the three natural allotetraploids, however, revealed that several quantitative and qualitative changes have taken place in their enzyme banding patterns. These changes in the natural allotetraploids would be expected to be more extensive than that of the recently synthesized allopolyploids due to the relative lengths of time involved for the evolutionary mechanisms to operate. Of interest at this point is a possible explanation of these evolutionary changes.

The combination of the genomes within an allotetraploid species can give the latter an advantage over each of the contributing diploid species; e.g., inactivation of specific genes within either of the two genomes by means of mutation would be overshadowed by the homeologous duplicated segments of the other genome. The latter would then continue to produce the original materials needed for development (Haldane 1932).
The theory of gene regulation in higher organisms, as proposed by Britten and Davidson (1969), provides a possible means of explaining the origin of species specific regulative systems through diploidization. These workers attempted to explain the unified control of non-contiguous genes that manipulate the production of chemical units not involved in genome regulation. Here, producer genes can be under the control of one or more regulatory genes. Thus, if the sequence of gene expression involved in seed development was under a specific control mechanism in an allotetraploid, a mutation could conceivably shut off the control mechanism in one or the other of the two genomes (further mutational changes in this selected control mechanism could then follow) and result in diploidization (Endrizzi 1966).

Differential quantitative and/or qualitative control of specific genes due to mutational changes within either one or the other homeologous genome, or in parts of both genomes of the natural allotetraploids, may be indicated by the different isozyme patterns of the three enzyme systems (Figures 12-14). However, these quantitative differences may also reflect significant differences in the dose of active genes (e.g., duplications) that are responsible for enzyme formation. Also, the differential availability of these enzymes in the dormant seeds to the extraction techniques cannot be discounted.

Genomes similar to $A_1$ and $D_5$ were also indicated by the superimposed banding patterns of seed proteins to be the most likely ancestors of the natural allotetraploids (Cherry, Katterman, and Endrizzi 1970). In addition, examination of the three natural allotetraploids
revealed that several changes had taken place in their protein composition. As observed for the enzyme data, the allopolyplploids that were synthesized recently showed little variation in their protein banding patterns when compared to the additive patterns of their parent species. Thus, these protein differences in the natural allotetraploids, as similarly observed for the three enzyme systems, further indicated that evolutionary processes are operating on both homeologous genomes of the polyploid species.

**Other Chemotaxonomic Studies Concerning the Genus *Gossypium***

Using infrared analysis of acetone-hexane-water extracts of defatted seed meal, El-Nockrashy, Simmons and Frampton (1969), observed that *G. tomentosum* differed from the other two allotetraploids; this difference suggested to them a biophyletic origin of the allotetraploids. The results of the studies with seed proteins and enzymes, on the other hand, agree with the conclusions derived from classical techniques and support a monophyletic or common ancestral origin of these three allotetraploids.

Other biochemical results concerning the relationships of the species within the genus *Gossypium*, i.e., nucleic acid composition (Ergle, Katterman, and Richmond 1964; Katterman and Ergle 1970) and amino acid analyses (Sarvella and Stojanovic 1968), also support the classical and electrophoretic studies.
SUMMARY

1. The techniques of polyacrylamide and starch gel electrophoresis were used to analyze the isozyme make-up of three enzyme systems (esterase, leucine aminopeptidase, and catalase) of dormant seeds from the genus Gossypium. Twenty-nine species and 29 varieties (including diploids and synthetic and natural allopolyplploids) were included in this phylogenetic study.

2. Examination of a large sample of seed from four populations of G. thurberi showed much isozyme polymorphism for esterase activity. Similar observations were also noted for limited seed samplings of other species within the genome groups. These data suggested that intraspecific variation should be evaluated before interspecific analyses were made. This variation was not observed for leucine aminopeptidase and catalase.

3. Species and/or varieties observed within each genome had banding patterns for the three enzyme systems that were more similar to one another than to members in other genome groups. In summary, only minor differences distinguished the A and B genome species, whereas band variations were greatest between more distantly related than closely related species in the C, D, and E genomes. The D genome, however, was further characterized by an increase in catalase activity. Gossypium longicalyx, formerly considered an E genome species, showed an overall banding pattern unique to itself. This difference indicated that it should be placed in a new genome, F. These results supported the
classification of the diploid species into the presently recognized six genome groups (A-F).

4. The frequency data from the esterase polymorphism of the isozyme patterns showed a specific relationship between species within the D and E genomes. These data showed that within a genome group certain zymograms occurred much more frequently in some species than in others. Those isozyme patterns occurring in low frequencies in the former, however, were more numerous in the latter species.

5. The species of the genomes (i.e., C, D, E, and F), removed from the postulated center of origin (South Africa) of the genus *Gossypium*, showed an increase in the amount of isozyme variability and major band activity over that produced by the suggested ancestor species (A and B genomes).

6. Synthetic mixtures of seed extracts from parent species of recently formed synthetic allopolyploids produced additive isozyme patterns for esterase, leucine aminopeptidase, and catalase. These additive patterns from the parent species were closely comparable to the zymograms produced by their polyploid progeny. These results indicated that similar analyses could be made with the natural allotetraploids (*G. tomentosum*, *G. barbadense*, and *G. hirsutum*) to determine their possible parent origin. Species containing the genetic make-up for the three isozyme systems similar to that produced by the A₁ and D₅ genomes were indicated to be the most likely ancestors of the natural allotetraploids. In addition, quantitative and qualitative differences distinguished the three natural allotetraploids. Diploidization and a recently proposed
regulation mechanism for higher organisms were discussed in relation to these isozyme differences.


