NORTH CAROLINA STATE COLLEGE
OF AGRICULTURE AND ENGINEERING
OF THE UNIVERSITY OF NORTH CAROLINA
OFFICE OF THE CHANCELLOR
RALEIGH

19 December 1957

Dr. Alan T. Waterman, Director
National Science Foundation
Washington 25, D. C.

Re: Research Grant NSF-G4851

Dear Dr. Waterman:

We are pleased to accept the research grant referred to above and acknowledge the conditions as stated in your letter of December 16, 1957. The grant will be administered according to these special conditions and in accordance with the general policies of the National Science Foundation as stated in "Grants for Scientific Research," April 1955.

We are grateful for this assistance, and I am confident that Drs. Gerstel and Phillips will conduct the project in a highly satisfactory manner.

Sincerely yours,

CHB

Carey H. Bostian
Chancellor

CHB:ho
cc: J. G. Vann
D. U. Gerstel

NATIONAL SCIENCE FOUNDATION
WASHINGTON 25, D. C.

DEC 16 1957

Dr. Carey H. Bostian, Chancellor
North Carolina State College
Raleigh, North Carolina

Research Grant NSF-G4851

Dear Dr. Bostian:

I am pleased to inform you that the sum of $17,000 is hereby granted by the National Science Foundation to North Carolina State College of Agriculture and Engineering, for the support of research entitled "Artificial Amphidiploids in the Genera Gossypium and Nicotiana," under the direction of D. U. Gerstel and L. L. Phillips, Department of Field Crops, for a period of approximately three years. Until further notice this grant will be paid as follows: $6,700 on or about two weeks from date of this letter; $5,600 on or about March 15, 1959; $2,800 on or about March 15, 1960; and $1,900 on or about September 15, 1960.

It is a condition of this grant that it may be revoked in whole or in part by the Foundation after consultation with the principal investigators and the grantee, except that a revocation shall not affect any commitment which, in the judgment of the Foundation and the grantee, had become firm prior to the effective date of the revocation; and that funds not committed by the grantee prior to the conclusion of the work contemplated under this grant shall be returned to the Foundation.

It is a further condition of this grant that disposition of domestic patent and other rights in any inventions or discoveries made or conceived during the research shall be the responsibility of the grantee; that disposition of foreign patent and other rights to any such invention or discovery shall be determined by the United States Government; that the grantee shall give the Foundation reasonable notice of application by the grantee or other person or institution for a foreign or domestic patent on any such invention or discovery; and that upon issue of a domestic patent on any such invention or discovery, the patentee shall grant the Government an irrevocable, royalty-free, nonexclusive license for use of such invention or discovery for governmental purposes.

The Foundation desires that this grant be administered in general accordance with the Foundation's policies for research grants as stated in "Grants for Scientific Research," April 1955, and in conformity with the other understandings reached between the Foundation and the grantee relating to this grant.

Please acknowledge receipt at your earliest convenience.

Sincerely yours,

Alan T. Waterman
Director

Dr Henry's copy
Cytogenetics - not to be

NORTH CAROLINA STATE COLLEGE

Department of Field Crops

Studies on the Segregation in Artificial Amphiploids in the Genus Gossypium

Final Report

September 1, 1957

combined with an application for continued support.

Grant No. G720

National Science Foundation

TABLE OF CONTENTS

1. Name and address of institution:

North Carolina State College, Raleigh, N. C.

2. Principal investigators:

Dr. D. U. Gerstel (grantee).

Dr. L. L. Phillips (joined the project in February, 1956).

3. Additional personnel who participated:

Graduate students: James B. Weaver, Jr.

Patricia A. Sarvella

Om P. Kamra

Technical assistants: Eloise Johansen (until June, 1955)

Joyce Burns (July 1955 until present)

4. Publications and manuscripts written under grant:

a. Journal papers and abstracts published.

1956

GERSTEL, D. U. Segregation in new allopolyploids of Gossypium. I. The R_1 locus in certain New World-Wild American hexaploids. Genetics 41: 31 - 44.

_____ The use of segregation ratios of synthetic allopolyploids as a taxonomic tool. J. Elisha Mitchell Scientific Society 72: 193. (Abstract).

_____ and P. A. SARVELLA. Additional observations on chromosomal translocations in cotton hybrids. Evolution 10: 408 - 414.

SARVELLA, P. A. Cytomixis in Cotton. J. Elisha Mitchell Scientific Society 72: 192. (Abstract)

1957

WEAVER, J. B., JR. Embryological studies following interspecific crosses in Gossypium. I. G. hirsutum x arboreum. Amer. J. Bot. 44: 209 - 214.

b. Journal papers in press:

GERSTEL, D. U. and L. L. PHILLIPS. Segregation in new allopolyploids of Gossypium. II. Tetraploid combinations. Genetics.

WEAVER, J. B. Embryological studies following interspecific crosses in Gossypium. II. G. arboreum x G. hirsutum Amer. J. Bot.

c. Journal papers in preparation:

GERSTEL, D. U. and L. L. PHILLIPS. Segregation in new allopolyploids of Gossypium. III. Leaf shape segregation in hexaploid hybrids of New World cottons.

_____ and _____. Genetic segregation in amphiploids synthesized from tetraploid cotton and diploid relatives.

KAMRA, O. P. Effects of polyploidy on cotton fibers.

SARVELLA, P. A. Cytomixis in cotton and related phenomena. (Submitted to Cytologia.)

_____. Multivalent formation and genetic segregation in some allopolyploid Gossypium hybrids.

d. Theses:

KAMRA, O. P. Effects of polyploidy on cotton fibers. Masters thesis, 1956.

SARVELLA, P. A. Multivalent formation and genetic segregation in some allopolyploid Gossypium hybrids with observations on cytomixis. Ph. D. thesis.

WEAVER, J. B. A study of embryo abortion in reciprocal interspecific crosses of Gossypium. Ph. D. thesis.

5. Description of accomplishments:

In summarizing the results of the last three years it is convenient to use the origin and the taxonomic position of G. hirsutum as a point of departure.

a. Genetics of amphiploids of G. hirsutum and its ancestral relatives.

This species which comprises the most widely grown cottons of New World origin is a natural amphiploid. Cytogenetic studies by Beasley, Skovsted and others performed in the nineteen thirties have left little doubt that 13 of the 26 pairs of chromosomes were derived from some Old World species of Gossypium closely allied taxonomically with the modern species G. arboreum and G. herbaceum. The remaining chromosomes came from an ancestor related to the diploid American species of which G. raimondii and G. thurberi are modern examples. Beasley has assigned the symbol A to the chromosomes of the Old World species, D to those of the Wild American species and, logically, he has designated the chromosomes of the amphiploids with AD. Evidence for the origin of the amphiploids derived mainly from Drosera type meiotic associations in triploid hybrids (table 1).

Table 1. Meiotic configurations in hybrids between G. hirsutum and relatives of its ancestral species.

F_1 Hybrid	A Chromosomes	D Chromosomes
G. hirsutum x thurberi	13_{I}	13_{II}
x raimondii	13_{I}	13_{II}
G. hirsutum x herbaceum	$9_{II} + {}^{2}IV$	13_{I}
x arboreum	$8_{II} + {}^{1}IV + {}^{1}VI$	13_{I}

F_1 G. hirsutum x raimondii quite regularly forms 13 II and 13 I and so does F_1 G. hirsutum x thurberi. In a similar way the 13 chromosomes of the D type remain unassociated in a G. hirsutum x Old World hybrid while the A type chromosomes form pairs, and, in this case, multivalents. Hybrids between G. hirsutum and G. herbaceum give 9 II and 2 IV; those of G. hirsutum and G. arboreum 8 II + 1 IV + 1 I VI. Thus, no microscopically visible rearrangements have occurred in the evolution of the D chromosomes but several segmental interchanges differentiate the A genomes (Gerstel and Sarvella, 1956). In view of these cytological facts, we were surprised when we got the genetical data contained in table 2.

Table 2. Gametic Ratios of Hexaploids.

Locus	Dominant	:	Recessive	Number of Plants
	6x G. hirsutum x Old World (AAAADD)			
Y	4.31	:	1	271
H	4.54	:	1	377
L_2	4.71	:	1	177
R_2	5.10	:	1	351
	6x G. hirsutum x G. raimondii (AADDDD)			
Cl	7.50	:	1	510
Cn	7.97	:	1	305
Yg	8.30	:	1	307
Cr	8.39	:	1	554
R_1	8.68	:	1	1297
Vy	9.86	:	1	554
L	11.00	:	1	602
Gl	16.18	:	1	653
	6x G. hirsutum x G. thurberi (AADDDD)			
R_1	46.71	:	1	334
L	47.86	:	1	342

These data were obtained by crossing newly synthesized hexaploids (i.e. 2AAD and 2ADD) as females with recessive testers. The hexaploids were, of course, duplex, i.e. RRrr, etc., for certain genes of the parent and testcrosses measured the output of gametes with and without dominant alleles.

Their proportion was determined by the extent of homology of the chromosomes on which they were located. If the chromosomes of the parent species were completely homologous 5:1 ratios were expected. Some decrease in the proportion of gametes carrying dominant genes could occur as a consequence of double reduction or of loss of chromosomes during meiosis.

The experimental data from G. hirsutum x Old World hexaploids gave a close fit to the 5:1 ratio; there was perhaps a slight tendency for the dominants to be deficient.

On the other hand, hexaploids synthesized from G. hirsutum and Wild American species produced fewer recessive carrying gametes - the Cl locus gave the closest approximation to a 5:1 ratio (s. table 2) but even here the difference is highly significant. Such reduction in segregation is likely to be the consequence of preferential association of G. hirsutum with G. hirsutum and Wild American with Wild American chromosomes in the hexaploid hybrids. If this sort of behavior were exclusive no segregation at all would result since all gametes would be of a single type. Neither type of hexaploid gave this extreme of complete absence of segregation which is characteristic of "true" allopolyploids. That, of course, is not at all expected because of the taxonomic relationships. But it is perhaps a little surprising to find that the chromosomes of the Old World species have retained full homology with those of the A genome of the New World, which is very surprising for the following reasons. The Old World species are cultigens extending along a wide belt from the Atlantic across Africa and Asia to the Pacific. They consist of innumerable races and varieties produced by human selection. The genetic lines used in the synthesis of

our hexaploids were obtained from modern, annual types of the two species which came from areas far removed from their presumed centers of origin. Hence, they are the most likely to have undergone great evolutionary changes. The direct comparison of the chromosomes supported this idea : the only chromosomal translocations which could be detected in the genus Gossypium were the three found in the A genome, as mentioned.

In contrast, the diploid species with D chromosomes are wild perennials of rather limited geographic ranges and presumably slower evolutionary pace. Their chromosomes have not changed visibly.

Since it is widely assumed that preferential association in an amphiploid is a consequence of differences in intimate chromosome structure, and if we admit that the amount of segregation is a quantitative measure of the extent to which this structure has changed during the course of evolution, we cannot but help being astonished to find so little differentiation in the A but much more of it in the D chromosomes.

> The data in table 2 suggest several questions which we cannot treat here in detail. A rough comparison of the three groups of data shows that all the chromosomes in a given type of hexaploid behave similarly; i.e., that the observed genetic ratios are characteristic for each of the hexaploids. Four different linkage groups are represented in the 6x G. hirsutum x Old World crosses, five or six different groups in the 6x G. hirsutum x raimondii crosses and two different chromosomes in 6x G. hirsutum x thurberi. Considerable differences are obvious in the middle group but these appear to be differences of degree. Further work is designed to discover how important these differences are and what their cause may be. In the meantime we are strongly impressed by the relative uniformity within the groups. This is rather unexpected if chromosomal structure changes during evolution by discrete steps of translocation, inversion, duplication, etc. As an alternative, with very little support at the present, one might suggest that differential affinity is not so much controlled by the structure of the chromosomes as by the genotype or the cytoplasm and therefore similar for all chromosomes in a polyploid hybrid.

Another question, and this concerns the validity of our data, is whether the results really reflect meiotic processes or whether the low number of recessives obtained was due to post-meiotic failure of gametes and zygotes. Fortunately, it was possible to recognize for certain genes three classes, formed from RR, Rr and rr type gametes (table 3); sometimes the RR and rr classes were of equal size which indicated that there was no discrimination against one of the classes.

Table 3. Examples of segregation where three classes could be recognized.

Parent	Gametic Output		
	"RR"	"Rr"	"rr"
6x G. hirsutum x raimondii Vy Vy vy vy	53	450	51
6x G. hirsutum x thurberi L_1 L_1 l_1 l_1	19	316	7

For example, segregation for Vy in hexaploid G. hirsutum x raimondii gave 53 VyVy : 450 Vyvy : 51 vyvy gametes. The most extreme difference between classes was afforded by the L_1 locus in 6x G. hirsutum x thurberi with a segregation of 19 L_1L_1: 7 l_1l_1. This result suggests that gamete elimination occurred; but in no case was gamete elimination of a sufficient magnitude to override the previously made generalization.

b. Genetics of other amphiploid combinations.

The genetics of several other synthetic amphiploids was also explored. The Wild African species G. anomalum has a morphology resembling that of the cultivated Old World species G. arboreum and G. herbaceum. On cytological grounds, however, this species had been assigned by Beasley the genome formula, B, distinct from the A_1 and A_2 of the Old World cultigens.

Table 4. Segregation of amphiploids with Wild G. anomalum.

Locus	Dominant	:	Recessive	Number of plants
	4x Old World x G. anomalum (AABB)			
R_2	111.00	:	1	112
Yc	89.20	:	1	451
Pf	81.20	:	1	411
Pa	67.50	:	1	411
RI_a	45.30	:	1	131
	4x G. anomalum x G. thurberi (BBDD)			
L^T	All	:	0	220
R_1	All	:	0	63
	6x G. hirsutum x G. anomalum (AABBDD)			
L^A	All	:	0	635
Bn	392.00	:	1	393
Lc	148.50	:	1	299
Fz	146.50	:	1	295

In table 4 are summarized the segregation data obtained from crosses of several amphiploid combinations of G. anomalum. They confirm the view that the chromosomes of the B genome are well differentiated from the A chromosomes since very little segregation occurred in the Old World x G. anomalum amphiploids for loci on five different chromosomes. Only two chromosomes were tested in the BBDD combination; both showed completely preferential pairing without segregation. Similarly very little segregation could be observed in hexaploids G. hirsutum x anomalum.

Table 5. Segregation data from amphidiploids of wild diploid species.

Locus	Dominant : Recessive	Number of plants
	4x G. arboreum x herbaceum ($A_1A_1A_2A_2$)	
RI_a	3.96 : 1	387
R_2	3.53 : 1	154
	4x G. raimondii x G.thurberi ($D_1D_1D_5D_5$)	
R_1	13.44 : 1	390
	4x G. arboreum x G. thurberi (AADD)	
R_2	All : 0	158
P_a	All : 0	118
L^N	117 : 1	118

In connection with the earliest discussion of amphiploids between G. hirsutum and its possible ancestral relatives, the behavior of amphiploids between these relatives is of interest (table 5). The results of testcrosses indicate that at least two chromosomes of G. arboreum which were tested have greatly similar G. herbaceum homologues. In this way additional confirmation was obtained that A chromosomes have not undergone any considerable cytological differentiation during their evolution. In contrast, the two D bearing sp ecies G. thurberi and G. raimondii show a rather low rate of segregation. As described above these two species had given very different results in hexaploid combinations with G. hirsutum. Unfortunately, only one chromosome could be tested.

c. Meiotic behavior of synthetic amphiploids.

A thorough cytogenetical analysis of the available amphiploids was

undertaken by Miss Sarvella and the results described in her doctoral thesis. The frequencies of multivalent chromosome associations (table 6) were found to be fairly closely correlated with the observed segregation ratios and their chromosomal basis was therefore ascertained.

Table 6. Average multivalent frequencies.

Amphiploid	Mean no. of Multivalents
G. hirsutum x anomalum	0.18
Old World x anomalum	1.90
G. hirsutum x thurberi	3.61
G. thurberi x raimondii	3.94
G. hirsutum x raimondii	6.16
G. arboreum x herbaceum	9.42

d. Endosperm development in interspecific crosses.

Since many interspecific Gossypium hybrids fail in the seed, Mr. J. B. Weaver undertook their study for his thesis problem. He found that when he used species with the lower chromosome number as the female parent the endosperm made rapid initial growth but degenerated 10-20 days after pollination without ever developing cell walls. The wider the chromosome ratio the more rapid was the degeneration. The embryo often reached 1/5 normal size but did not differentiate properly. Maternal tissues of the ovule developed in an almost normal manner.

In reciprocal crosses, except for 6n x 4n, development was quite different. The endosperm formed cell walls prematurely and ceased growth after five days while the embryo grew at a normal rate for the first 10 days and then growth lagged. The maternal tissue stopped growth

immediately after the endosperm. However, when the egg cell was not fertilized the endosperm grew at a normal rate and a large embryoless ovule was produced. This indicates that the hybrid embryo causes some physiological unbalance which inhibits endosperm growth when both develop within the same ovule.

e. Influence of polyploidy on cotton fibers.

Since the requisite plant materials had become available through the experiments described above, Mr. O. P. Kamra decided to utilize them for a Master's of Science project on the influence of polyploidy on cotton fibers. While the effects of artificial polyploidization on cell dimensions are well known in many organisms, this has not yet been investigated in the case of the cotton fiber cell. But of even greater practical and theoretical interest was the question of the effect of polyploidization on the structure of the cell wall, which determines the worth of the fiber. Mr. Om studied therefore both the effects of doubling of the chromosomes on fiber perimeters and on the x-ray angles of the cellulose deposit. Artificial polyploids of New World cottons (Gossypium hirsutum and G. barbadense) and Old World species (G. herbaceum, G. arboreum and their F_1 hybrid) were utilized. Some of the treated plants were chimerical affording an opportunity to determine the developmental relation between the lint bearing seed epidermis and the leaf epidermis. In some individuals seed and leaf epidermis were both found to be doubled, while in other cases only one or the other had polyploid features, suggesting their derivation from distinct germ layers. Fiber perimeters were significantly increased in Old World tetraploids as compared with their diploids. In G. hirsutum

octoploids the fiber perimeter was in most cases not increased. This was ascribed to a chimerical condition of the plants rather than to an upper limit for cell size since 8n G. barbadense fibers did have an enlarged perimeter and one such case was also found among the G. hirsutum octoploids. For any particular genotype and the derived polyploids the data revealed distinctly proportional changes of x-ray angle and mean perimeter, leaving the number of helical turns of the cellulose deposit uniform per unit length of fiber. On the other hand, since the length of the helical turn varied from species to species, and has been subjected to selective change within a species, it is tentatively suggested that this characteristic is directly dependent upon the genotype and not upon the number of chromosomes.

6. Title of proposed continued research:

   Studies on the segregation in artificial amphidiploids in the genera Gossypium and Nicotiana.

7. Desired starting date and time period for which support is requested:

   February 1, 1958; to run for three years.

8. Principal joint investigators for proposed continued research:

   Dr. D. U. Gerstel

   Dr. L. L. Phillips

9. Description of proposed continued research:

   Since Dr. L. L. Phillips has joined our laboratory in 1956, it is possible to attack on a broader scope the problems which have opened up during the past triennium. It is planned to continue work with

Gossypium along the lines described below and to experiment also with the genus Nicotiana. While close coordination will be maintained, Dr. Phillips will be responsible for the Gossypium work and Dr. Gerstel for Nicotiana.

a. Gossypium

The work of the past three years has suggested, at least for the genus Gossypium, a good correlation between taxonomic affinities on the one hand and preferential chromosome pairing on the other. Since only a few chromosomes were tested in each synthetic amphiploid the work should be expanded to include markers on additional chromosomes. This should serve to provide an answer to the focal problem: Do genes on different chromosomes of a given amphiploid segregate with the same frequencies or does each chromosome have a specific preferential affinity for the "homologues" from the other species and produce therefore specific segregation rates? From a solution of this problem should ultimately emanate new insights into the nature of preferential pairing in polyploids and chromosome differentiation in evolution.

1. Additional markers have been incorporated in synthetic polyploids; since several of these are seedling markers it should be possible to accumulate large populations for sensitive statistical tests. It will be possible then, in favorable cases, to decide whether differences like those encountered in the middle group of table 2 are significant or not.
2. In order to test the possibility that the genotype of a plant, rather than individual chromosome structure, determine

preferential pairing octoploids of the type AAA'A'DDD*D* have been synthesized which will segregate simultaneously on the A and D genomes. If segregation for A and D genome factors on one and the same genetical background should prove to be similar, a genotypic influence on chromosome pairing would be indicated, since it differed so widely in the separate AADDDD and AAAADD hexaploids (table 2).

3. The relations of only two of the six Wild American (D) species to the New World (AD) species G. hirsutum and, to a lesser extent, to G. barbadense have been thus far studied. Since neither G. thurberi nor G. raimondii were found to carry chromosomes very similar to the New World D genomes it is of interest to introduce other D species into the program and to test whether they might be more closely related to the New World species. A number of hexaploid combinations of various Wild American and New World species have become available.

Studies have also been initiated on the segregation of octoploid hybrids of the three New World species G. hirsutum, G. barbadense and G. tomentosum.

b. Nicotiana:

It appears desirable to investigate whether the pattern of amphiploid behavior discovered in Gossypium also apply to another genus. For this purpose Nicotiana was chosen for a number reasons. Nicotiana, like Gossypium, contains several thoroughly analyzed natural amphiploids; this will permit the synthesis and subsequent analysis of higher polyploids containing four more or less closely related genomes, as in Gossypium (see point 2., above). Nicotiana also is a larger genus,

with about 55 species, permitting the production of a great number of hybrids; from such hybrids amphidiploids can be produced with a wide range of taxonomic relationships between the parent species. Nicotianas also give abundant seed with a minimum of handling. This will permit a more accurate statistical exploitation of the results. The main drawback of Nicotianas are their great susceptibility to diseases and the pronounced contraction of the chromosomes at meiosis which is an obstacle to multivalent analysis.

An almost complete collection of Nicotiana species and a considerable number of N. tabacum marker genes (about 25) have recently become available at North Carolina State College; also, one of the applicants (Gerstel) has had some experience with that genus. A few amphidiploid combinations (N. tabacum x tomentosiformis, N. tabacum x otophora, N. tabacum x sylvestris, N. rustica x paniculata, N. rustica x knightiana, etc.) have already been synthesized, involving various marker genes of N. tabacum and N. rustica. The first segregating populations are in the 1957 field.

It is planned to apply similar procedures to this genus as had been used with Gossypium.

10. Personnel:

Dan Ulrich Gerstel

Born October 23, 1914 at Berlin-Dahlem, Germany.
Received secondary education at a Berlin Gymnasium.
Attended Universities of Grenoble, France; and Fribourg, Switzerland.
Received B.S., M.S., and Ph. D. (1945) at University of California at Berkeley, where he also served as Teaching fellow in Botany and Associate in Genetics.
Associate Geneticist of the Guayule Project for U.S.D.A. from 1947-50.
At N. C. State College since April, 1950. Now Professor of Field Crops.
Member of Genetics Society, Evolution Society, and American Naturalists.

Bibliography (not listed are papers written under NSF grant G720).

1943 Inheritance of Nicotiana Tabacum. XVII. Cytogenetical analysis of glutinosa-type resistance to mosaic disease. Genetics 28:533-536.

1945 Inheritance in Nicotiana Tabacum. XIX. Identification of the Tabacum-chromosome replaced by one from N. glutinosa in mosaic resistant Holmes Samsoun tobacco. Genetics 30:448-454.

Inheritance in Nicotiana Tabacum. XX. The addition of Nicotiana glutinosa chromosomes to tobacco. Jour. Heredity 34:197-206.

1946 Inheritance in Nicotiana Tabacum. XXI. The mechanism of chromosome substitution. Genetics 31:421-427.

1947 "Cytogenetics of the Genus Nicotiana" by D. Kostoff, review in Quart. Rev. Biol. 22:333.

1948 A cytological investigation of tetraploid Rhoeo discolor. Am. Jour. Bot. 35:141-150. (with M. S. Walters).

Transfer of the mosaic resistance factor between H-chromosomes of Nicotiana glutinosa and N. Tabacum. Jour. Agric. Res. 76:219-223.

1949 Hydrochloric acid as a fixative for root tip chromosomes. Stain Technology 24:95-97.

1950 Self-incompatibility studies in Guayule: I. Pollen tube behavior. Jour. Heredity 41:49-55. (with M. E. Riner).

Self-incompatibility studies in Guayule: II. Inheritance. Genetics 35:482-506.

Is resistance to Verticillium wilt in guayule related to chromosome number? Agronomy Journal 42:310-311.

On the inheritance of apomixis in guayule. Botanical Gazette 112:96-106. (with William Mishanec).

On the effect of species-foreign pollen on self-incompatible guayule. Genetics 35:666 (Abstract).

1953 An additional note on the inheritance of apomixis in Parthenium argentatum. Botanical Gazette 115:89-93. (with B. L. Hammond and C. Kidd).

Chromosomal translocation in interspecific hybrids of the genus Gossypium. Evolution 7:234-245.

On the influence of temperature on a genetic ratio. J. Elisha Mitchell Scientif. Sci. 69:84 (Abstract).

Fingernail lacquer as a sealing medium for cytological squashes. Turtox News 31:54.

Genetic segregation of allopolyploids in the genus Gossypium. Genetics 38:664-665 (Abstract).

1954 A new lethal combination in interspecific cotton hybrids. Genetics 39:628-639.

1955 A pollen mother cell of Asiatic cotton with only ten pairs of chromosomes. Cytologia 20:197-198.

Lyle Llewellyn Phillips

Born June 14, 1923 at Long Beach, California.
Received B.A. from the University of Redlands 1950; M.A. from Claremont College 1951; Ph. D. from University of Washington, 1954.
Teaching Fellow, University of Washington, Seattle, 1951-1954.
Post-doctoral Fellow, Washington State College, Pullman, 1954-1956.
Assistant Professor of Field Crops at N. C. State College, since 1956.

Bibliography (exclusive of papers under 4., above).

1955 A revision of the perennial species of Lupinus of North America. Res. Stud. of State Coll. Wash. 23:161-200.

1956 Effect of free radicals on chromosomes of barley. Science 124:889-890.

Chromosome numbers in Lupinus. Madrono 14:30-36.

1957 The effects of fractional doses on X-rays of dormant barley seeds. Northwest Science 31:80-91. (with A. B. Schooler and R. A. Nilan).

II. Facilities:

Available on Campus is a large airconditioned cytogenetics laboratory with adjacent offices in the Field Crops Department. These facilities

are sufficient to accomodate two investigators and several graduate students or assistants (Rental value $4,100. p.a.). Four good microscopes with the necessary additional equipment are on hand. Two separate greenhouses are available for the cotton and tobacco work (Rental value $3,000. p.a.). Field space of several acres with the required equipment and labor for field operations ($900. - for rental and operation cost p.a.), in proximity of the Campus will be used.

12. Proposed Budget:

		First Year		Second Year		Third Year	
		State	NSF	State	NSF	State	NSF
a/	Salaries						
	Prin. Invest.						
	D. U. Gerstel -- 1/2 sal.	$4,500	-	$4,500	-	$4,500	-
	L. L. Phillips -- 3/4 sal.	4,950	-	4,950	-	4,950	-
	3 Graduate Students		$6,000		$6,000		$6,000
b/	Facilities (Item #11)	8,000	-	8,000	-	8,000	-
c/	Permanent Equipment						
	Misc. Equipment	200	200	200	-	200	-
d/	Expendable Equipment and Supplies	700	700	700	700	700	700
e/	Travel	300	100	300	100	300	100
f/	Other Direct Costs						
	Contractual including Photography	100	100	100	100	100	100
g/	Overhead	-	1,065	-	1,035	-	1,035
		$ 18,750	8,165	18,750	7,935	18,750	7,935

Total supplied by State in three year period $56,250

Total requested for three year period $24,035

13. Approval:

D. U. Gerstel Principal Coinvestigator	J. G. Vann Business Manager
L. L. Phillips Principal Coinvestigator	R. L. Lovvorn, Director of Research Agricultural Experiment Station
P. H. Harvey, Head Department of Field Crops	Carey H. Bostian Chancellor

National Science Foundation
NSF Form 612, October 1952

Form Approved
Budget Bureau No. 99-R013

NATIONAL SCIENCE FOUNDATION

GRANT FISCAL REPORT

From: Dr. D. U. Gerstel
(Grantee)
N. C. State College, Raleigh, North Carolina
(Address)

To: Chief Grants Administrator
NATIONAL SCIENCE FOUNDATION
Washington 25, D. C.

Date: July 1, 1957

Report of obligations from NSF funds for grant number: _____ (NSF No.)

Reporting period: December 30, 1956 (Date) to June 30, 1957 (Date) (inclusive)

Type report: Interim [ ], Number ___; final [X] (check one)

	Item	Amount Expended
1.	Salaries and wages	$ 1,136.63
2.	Equipment (permanent)	0
3.	Supplies, materials, and expendable equipment	0
4.	Travel	0
5.	Publication costs (Total - page costs, reprints, direct labor, etc.)	0
	(a) Page costs only, if available	
	(b) Reprints, direct labor, and any other publication costs	
6.	Other (specify):	0
		0
7.	Total direct costs - Add lines 1 through 6	1,136.63
8.	Allowance for indirect costs - ____% of line 7	0
9.	Total obligations for above period - Add lines 7 & 8	1,136.63
10.	Total obligations for prior periods	11,363.37
11.	Total obligations to date - Add lines 9 & 10	12,500.00

COMMENTS: (Continue on reverse side if necessary)

"I certify that this final fiscal report is correct and that the expenditures included herein are deemed properly chargeable to the grant"

SIGNED: D. U. Gerstel TITLE: Proffessor

TYPE NAME: D. U. Gerstel

Two copies of an interim fiscal report are due approximately six months from award date of a grant and at six-month intervals thereafter. Firm outstanding commitments should be considered as obligations for the purposes of interim reports. Two copies of a final fiscal report are due as soon as possible after all costs chargeable to the grant are known. The following certification, in the case of final reports, should be inserted above, immediately preceding the signature of the official authorized to sign for the grantee institution: "I certify that this final fiscal report is correct and that the expenditures included herein are deemed properly chargeable to the grant." Unused funds are to be returned by check payable to the National Science Foundation.

GPO 83-44886

ANNUAL REPORT TO THE NATIONAL SCIENCE FOUNDATION

Grant No. G720 - Calender Year 1956

TITLE: Studies on the segregation in artificial amphiploids in the genus Gossypium.

PRINCIPAL INVESTIGATOR AND INSTITUTION: D. U. Gerstel, North Carolina State College

PRINCIPAL RESULTS OF THE YEAR: In the preceding years we found that segregation ratios in artificial amphiploids often ranged between the two possible extremes of mendelian tetrasomic segregation and no segregation at all. Two questions of theoretical interest resulted from these observations: 1) do all thirteen groups of chromosomes present in quadruplicate in an amphiploid of the genus Gossypium (n= 13) behave alike with respect to conjugation and segregation, and 2) are the phenotypic frequencies among the offspring of an amphiploid real reflections of meiotic behavior or are they biased by differential mortalities of gametes and zygotes?

The first question could be studied in five different polyploid combinations in which gene contrasts at several loci had been produced by combining two dominant genes from one and two recessive alleles from the other parent species. Thus, tetraploid Old World cotton x G. anomalum amphiploids gave a mean ratio of 176.4 : 1 for eight loci. Segregation for each individual could be fitted to the total, but because of the wide segregation ratio the statistical test was very obtuse. Actually, no segregation occurred for leaf shape (635 : 0); this observation might prove of significance. (With deviations from the norm as rare as in this material one might doubt at all whether the deviants were due to segregation, or rather to misdistribution of chromosomes or mutational events. In any case, stability was extremely high for all loci tested).

Amphiploid G. hirsutum x G. raimondii (= 2 ADD in terms of a genomic formula) gave segregation ratios of much lesser width varying from 7.9 : 1 to 16.2 : 1 for a series of nine loci (some linked). The differences between the extremes were significant. This could have been due to divergent behavior of the various groups of homologous chromosomes or to differential mortality of the products of segregation (s. below).

For the fourth combination, G. hirsutum x Old World cotton, preliminary results for one locus were reported last year with a measure of surprise. This 2 AAD combination segregated like an autopolyploid in marked contrast with the wider segregation ratio of the 2 ADD combinations. Similar results were again obtained this year with four independent loci; for each of these a mendelian tetrasomic ratio was observed.

The last amphidiploid tested, 4n G. arboreum x G. herbaceum, gave a fit to a mendelian tetrasomic ratio for five independent loci.

Occurrence of selective mortality could be tested in various ways. In certain cases with incomplete dominance offspring from three types of gametes (AA, Aa and aa) produced by an AAaa amphidiploid were distinguishable. In several such instances gametes carrying two alleles (of the type AA) from one parent were not recovered with the same frequency as gametes carrying two alleles (aa) from the other parent. This suggests the occurrence of gametic or zygotic selectivity. Another kind of observation points in the same direction. The leaf shape and crinkle genes of G. hirsutum are linked, yet G. hirsutum x G. raimondii amphiploids gave rather divergent segregation ratios for the two factors. For a third observation, reciprocal crosses using the same amphiploid as male as well as female parent could be performed in a few instances. In these cases the reciprocal crosses gave similar results.

In summary, the two questions put above may be answered tentatively in the following way: It looks as if in general each amphiploid has its own characteristic segregation ratio. This segregation ratio is a reflection of the taxonomic relationship of the two parent species used in compounding the amphiploid. Differences in segregation frequency between chromosomes in the same amphiploid could be observed in some instances, but these differences might well turn out to be due to gametic or zygotic elimination.

PUBLICATIONS AND MANUSCRIPTS:

GERSTEL, D. U. Segregation in new allopolyploids of Gossypium. I. The R_1 locus in certain New World-Wild American hexaploids. Genetics 41:31-44, 1956

-------- The use of segregation ratios of synthetic allopolyploids as a taxonomic tool. J. Elisha Mitchell Scientific Society 72:193, 1956. (Abstract).

GERSTEL, D. U. and P. A. SARVELLA. Additional observations on chromosomal translocations in cotton hybrids. Evolution 10: (In press).

GERSTEL, D. U. and L. L. PHILLIPS. Segregation in new allopolyploids of Gossypium. II. Tetraploid combinations. (Manuscript prepared)

--------- Segregation in new allopolyploids of Gossypium. III. Leaf shape segregation in hexaploid hybrids of New World cottons. (Manuscript in preparation).

SARVELLA, P. A. Multivalent formation and genetic segregation in some allopolyploid Gossypium hybrids with observations on cytomixis. (Ph.D. thesis, N. C. State College Library, deposited June 1956).

--------- Multivalent formation and genetic segregation in some allopolyploid Gossypium hybrids. (Manuscript prepared).

--------- Cytomixis in the genus Gossypium. (Manuscript in preparation).

OES—Form 8
(June 6, 1950)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1955

(Three copies to be given to the OES examiner)

1. PROJECT: (Fund, number, and title): B-J Sec. 9, 9(S-1) THE COMPARATIVE CYTOGENETICS OF UPLAND COTTONS AND RELATED DIPLOID SPECIES

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Agronomy, N. C. State College; Division of Cotton and Other Fiber Crops, U.S.D.A.; and National Science Foundation.

3. PERSONNEL: D. U. Gerstel (project leader), Patricia A. Sarvella and J. B. Weaver (Graduate assistants) and Eloise Inmon (technical assistant).

4. NATURE OF RESEARCH AND PRINCIPAL RESULTS OF THE YEAR (Confidential information should be so marked): The barriers which separate the various cotton species and limit the production of new combinations of their germ plasm are being studied by means of genetical, cytological and embryological techniques. Genetically, segregation of marker genes in synthetic polyploid hybrids is being studied and findings to-date indicate fairly uniform behavior of different genes in any combination between two species but great differences between combinations. These range from almost total absence of recombination to the theoretically possible maximum. Cytological studies revealed partial, but incomplete correlation between multivalent chromosome associations and the genetical differences. Embryological work emphasized the details of the breakdown of embryo and endosperm in hybrids between Asiatic and American cultivated cottons and revealed consistent differences between reciprocal crosses of the two species. Comparisons of 4n x 2n, 6n x 4n and 2n x 4n and 4n x 6n crosses suggest that success and failure of crosses are largely determined by chromosome numbers.

Facilities used were about 1 acre of experimental plots, greenhouse space about 30 x 40 feet and cytological and embryological laboratory facilities and space.

5. APPLICATION OF FINDINGS (expressed in terms of measurable public benefits if and when justified): The utilization of wild species in a breeding program requires understanding of the barriers which separate them from the cultivated cottons. The project is directed towards discovering the barriers and determining the proper crosses and other means to overcome them.

6. WORK PLANNED FOR NEXT YEAR: As the work deals with problems of great theoretical complexity it is not intended as yet to make any changes in direction but rather to widen and deepen the scope by testing a widening array of marker genes and enlarging the present cytological and embryological data. However a number of new hybrids, some of which have never been made before, have also been produced for study.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

GERSTEL, D. U. Genetic segregation of allopolyploids in the genus Gossypium. Genetics 38:.- A new lethal combination in interspecific cotton hybrids. Genetics 39:.- The theory and practice of the back-cross method in the breeding of some non-cereal crops. By Mary Thomas. (Book review). Quart. Rev. of Biol. 29:.

Weaver, J. B., Jr. Reciprocal grafts between genetically lethal and normal cotton. J. Elisha Mitchell Scientif. Soc. 70.

8. Prepared by D.U. Gerstel Approved _____ (Director).

Date February 23, 1955 Date _____

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION PROJECTS

1. PROJECT: (Fund, number, and title):

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Agronomy, N. C. State College; Division of Cotton and Other Fiber Crops, U.S.D.A.; and National Science Foundation.

3. PERSONNEL: D. U. Gerstel (project leader), Patricia A. Sarvella and J. B. Weaver (Graduate assistants) and Eloise Johansen (technical assistant).

4. NATURE OF RESEARCH AND PRINCIPAL RESULTS OF THE YEAR (Confidential information should be so marked): The barriers which separate the various cotton species and limit the production of new combinations of their germ plasm are being studied by means of genetical, cytological and embryological techniques. Genetically, segregation of marker genes in synthetic polyploid hybrids is being studied and findings to-date indicate fairly uniform behavior of different genes in any combination between two species but great differences between combinations. These range from almost total absence of recombination to the theoretically possible maximum. Cytological studies revealed partial, but incomplete correlation between multivalent chromosome associations and the genetical differences. Embryological work emphasized the details of the breakdown of embryo and endosperm in hybrids between Asiatic and American cultivated cottons and revealed consistent differences between reciprocal crosses of the two species. Comparisons of 4n x 2n, 6n x 4n and 2n x 4n and 4n x 6n crosses suggest that success and failure of crosses are largely determined by chromosome numbers.

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7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

8. Prepared by D.U. Gerstel Approved _____ (Director).

Date February 23, 1955 _____

Draft

A PROPOSAL

TO

NATIONAL SCIENCE FOUNDATION

for Support of Research Entitled

Investigations on Genetic Instability in Nicotiana

Covering the period from March 1, 1962 through April 30, 1965

SUBMITTED BY

D. U. Gerstel, Principal Investigator
Professor of Field Crops
Department of Field Crops
North Carolina State College

P. H. Harvey, Head
Department of Field Crops
North Carolina State College

John T. Caldwell, Chancellor
N. C. State College of Agriculture
and Engineering of The
University of North Carolina
Raleigh, N. C.

W. D. Charmichael, Jr., Vice-President
and Finance Officer
Consolidated University of North
Carolina
Chapel Hill, N. C.

NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING
OF THE UNIVERSITY OF NORTH CAROLINA
Raleigh, North Carolina

1. Name and address of institution

North Carolina State College, Raleigh, North Carolina

2. Title of the proposed research

Investigations on genetic instability in Nicotiana

3. Description of proposed research

TO BE SUPPLIED

4. The principal investigator

Dan Ulrich Gerstel

Born October 23, 1914 at Berlin-Dahlem, Germany.

Received secondary education at a Berlin Gymnasium.

Attended Universities of Grenoble, France; and Fribourg, Switzerland.

Received B.S., M. S. and Ph.D. (1945) degrees at the University of California at Berkeley, where he also served as Teaching Fellow in Botany and Associate in Genetics.

Associate Geneticist of the Guayule Project for U. S. D. A. from 1947-1950.

At N. C. State College since 1950. Now Professor of Field Crops.

Lecturer in Summer Institute in Genetics for College Teachers (N. S. F. sponsored). Summer 1960

Visiting Professor at the Weizmann Institute of Science 1961-1962.

Member of Genetics Society, Evolution Society, American Naturalists and North Carolina Academy of Sciences.

Naturalized U. S. citizen December 1943.

Bibliography:

1943 Inheritance in Nicotiana Tabacum. XVII. Cytogenetical analysis of glutinosa-type resistance to mosaic disease. Genetics 28: 533-536.

1945 Inheritance in Nicotiana Tabacum. XIX. Identification of the Tabacumchromosome replaced by one from N. glutinosa in mosaic resistant Holmes Samsoun tobacco. Genetics 30: 448-454.

Inheritance in Nicotiana Tabacum. XX. The addition of Nicotiana glutinosa chromosomes to tobacco. Jour. Heredity 34: 197-206.

1946 Inheritance in Nicotiana Tabacum. XXI. The mechanism of chromosome substitution. Genetics 31: 421-427.

1947 "Cytogenetics of the Genus Nicotiana" by D. Kostoff. Review in Quart. Rev. Biol. 22: 333

National Rubber Research Project. Final Report (Mimeo.). Stanford University. (With R. C. Rollins and D. G. Catcheside)

A report on the morphological variation in Tau-saghyz with a plan for seed collections. Stanford Research Institute (Mimeo.).

1948 A cytological investigation of tetraploid Rhoeo discolor. American Jour. Botany 35: 141-150. (With Martha S. Walters).

Transfer of the mosaic resistance factor between H-chromosomes of Nicotiana glutinosa and N. tabacum. Jour. Agricultural Research 76: 219-223.

1949 Hydrochloric acid as a fixative for root-tip chromosomes. Stain Technology 24: 95-97.

1950 Self-incompatibility studies in guayule. I. Pollen tube behavior. Jour. Heredity 41: 49-55. (With M. E. Riner)

Self-imcompatibility studies in guayule. II. Inheritance. Genetics 35. 482-506.

Is resistance to Verticillium wilt in guayule related to chromosome number? Agronomy Jour. 42: 310-311.

On the inheritance of apomixis in guayule. Botanical Gazette 112: 96-106. (With M. Mishanec)

On the effect of species foreign pollen on self-incompatible guayule. Genetics 35: 666. (Abstract)

Biosynthesis of rubber in guayule and related investigations. Calif. Institute of Technology. (Mimeo.). (With J. Bonner and B. Arrequin)

1953 Chromosomal translocation in interspecific hybrids of the genus Gossypium. Evolution 7: 234-245.

On the influence of temperature on a genetic ratio. Jour. Mitchell Scientific Society 69: 84. (Abstract)

An additional note on the inheritance of apomixis in Parthenium argentatum. Botanical Gazette 115: 89-93. (With B. L. Hammond and C. Kidd)

Fingernail lacquer as a sealing medium for cytological squashes. Turtox News 31: 54.

Genetic segregation of allopolyploids in the genus Gossypium. Genetics 38: 664-665. (Abstract)

1954 A new lethal combination in interspecific cotton hybrids. Genetics 39: 628-639.

1955 A pollen mother cell of Asiatic cotton with only ten pairs of chromosomes. Cytologia 20: 197-198.

1956 Segregation in new allopolyploids of Gossypium. I. The R_1 locus in certain New World-Wild American hexaploids. Genetics 41: 31-44.

The use of segregation ratios of synthetic allopolyploids as a taxonomic tool. Jour. Mitchell Scientific Society 72: 193.

Additional observations on chromosomal translocations in cotton hybrids. Evolution 10: 408-414. (With P. Sarvella)

1957 Segregation in new allopolyploids of Gossypium. II. Tetraploid combinations. Genetics 42: 783-797. (With L. L. Phillips)

1958 Segregation of synthetic amphiploids in Gossypium and Nicotiana. Cold Spring Harbor symposia on Quantitative Biology 23: 225-237. (With L. L. Phillips).

1959 Segregation in new allopolyploids of Gossypium. III. Leaf shape segregation in hexaploid hybrids of New World cottons. Jour. Heredity 50: 103-108. (With L. L. Phillips)

Segregation in raw allopolyploids of Nicotiana. Genetics 44: 513. (Abstract).

1960 Controlled introgression in Nicotiana: A cytological study. Tobacco Science 151: 147-150. (With L. G. Burk).

Segregation in new allopolyploids of Nicotiana. I. Comparison of 6x (N. tabacum x tomentosiformis) and 6x (N. tabacum x otophora). Genetics 45: 1723-1734.

1961 Genetic instability in polyploid hybrids between tobacco and its ancestral relatives. Jour. Mitchell Scientific Society (Abstract: in press).

On chromosomal control of preferential pairing in Nicotiana. Science (In press).

Essay on the origin of tobacco. Tobacco Science. (In press).

Additional information on the mechanism of chromosome substitution. Tobacco Science (In press: with K. A. Patel).

The origin of tobacco: new problems. Bull. Res. Coun. Israel (Abstract: In press).

A haploid of Nicotiana tabacum deficient for two chromosomes. (Prepared: with L. G. Burk.)

5. Facilities

Available on campus is a large air-conditioned tobacco cytogenetics laboratory in the Field Crops Department. This consists of three rooms and is sufficient to accommodate the investigator and several graduate students and assistants. One of the rooms is being shared with cotton cytogenetics. Four good research microscopes with the necessary additional equipment for microphotography are on hand. There is ample greenhouse space on campus and field space of several acres is available for tobacco cytogenetics work at a distance of about 5 miles from the campus. The necessary equipment and labor for field operations can be obtained as required.

6. Proprosed budget

	First Year		Second Year		Third Year	
	NSF	NC State and others	NSC	NC State and others	NSC	NC State and others
Salaries						
Principal Investigator 75%	------	8,000	------	8,000	------	8,000
Cyto-technician	2,000	2,300	2,000	2,300	2,000	2,300
Graduate Assistants	2,700	2,700	2,700	2,700	2,700	2,700
Greenhouse Assistant	2,000	2,000	2,000	2,000	2,000	2,000
Secretarial Help	500	500	500	500	500	500
Labor	400	200	400	200	400	200
Equipment and Supplies	700	700	700	700	700	700
Communications	100	100	100	100	100	100
Travel	200	100	600	100	200	100
Contractual and Printing	200	100	200	100	200	100
	8,800	16,700	9,200	16,700	8,800	16,700
Overhead 20%	1,760	------	1,840	------	1,760	------
	$ 10,560	$ 16,700	$ 11,040	$ 16,700	$ 10,560	$ 16,700

Total request for 3 year period $ 32,160

7. Explanation of Budget Items

Salaries

1) Cyto-technician. The cytotechnician will make routine cytological preparations and anatomical sections. She will also assist in preparing plantings, routine statistical calculations and keeping scientific records.

2) Graduate Assistant. A half-time research assistantship in support of advanced degree program.

3) Greenhouse Assistant. This assistant will be in charge of the culture and maintenance of the greenhouse plantings and will assist in scoring and making crosses in the adjacent garden.

4) Secretary. A considerable portion of the secretary's time has been used in this program in the past and should be recognized in the present proposal.

Labor

1) This item is included for hired labor employed for field operations like ploughing, planting, pest control and weeding.

Equipment and Supplies

While no larger expenses for equipment are anticipated, smaller non-expendable items may be needed during the course of the investigations. The item is designed, in the main, to be used for routine supplies forthe cytological laboratory as well as for greenhouse and field items as insecticides, fertilizer, plant containers, labels, etc.

Communications

Item for telephone and occasional wire expenses, as well as postage; among the latter the largest proportion will go for the shipment of reprints.

Travel

Item for travel to and from national meetings and symposia. Travel to the International Congress of Genetics to be held in 1963 is anticipated for the second year.

Contractual and Printing

This item will cover such expenses as photography, gasoline and other automobile expenses, and cost of reprints.

ANNUAL REPORT TO THE NATIONAL SCIENCE FOUNDATION

Grant No. G-4851 January 1959-January 1960

TITLE: Studies on the segregation in artificial amphidiploids in the genera Gossypium and Nicotiana.

PRINCIPAL INVESTIGATORS AND INSTITUTION: D. U. Gerstel and L. L. Phillips, North Carolina State College, Raleigh, N. C.

ADDITIONAL PERSONNEL: Joyce A. Burns (Laboratory Technician), M. Alice Strickland (Laboratory Technician), and Shun-Jun Yang (Graduate student).

PRINCIPAL RESULTS:

The segregation data of this report, as those of previous ones, are based on testercrosses of the type AAaa x a, where AAaa represents an amphiploid with A introduced from one parent species, A derived from the other component species of the amphiploid and a a recessive tester which is in most instances recessive type Gossypium hirsutum in the cotton material and recessive Nicotiana tabacum for the tobaccos. As discussed previously in detail, a small segregation ratio (e.g. 5 A : 1 a phenotypes) is taken as evidence of close homology of the chromosomes carrying A and a, whereas a large ratio (e. g. 20 A : 1 a) indicates a much reduced homology and consequently a more remote taxonomic relationship.

GOSSYPIUM

Additional segregations have been scored for several hexaploid amphidiploids for which only tentative values, based on rather small segregating populations, were cited in last year's report. Also segregation ratios are available from one new hexaploid type combining G. barbadense with one of the Wild American diploid species, G. gossypioides. This hexaploid gave segregation ratios wider than any other New World x American diploid hybrid yet studied. Average segregation for three loci was 61.2:1.

For the purpose of comparison, the segregations for leaf-shape and anthocyanin coloration as well as the average segregation for all loci scored

in the New World x Wild American hexaploids thus far studied are presented below (data for the G. raimondii and G. thurberi hexaploids previously reported):

	Segregation	
	L and R	Ave. for all loci
6x G. hirsutum x raimondii	L 11.2 : 1 R 9.5 : 1	9.3 : 1
6x G. hirsutum x armourianum	L 13.6 : 1 R 24.9 : 1	15.9 : 1
6x G. hirsutum x harknessii	L 11.5 : 1 R 23.4 : 1	16.4 : 1
6x G. hirsutum x lobatum	L 16.5 : 1 R 27.9 : 1	20.0 : 1
6x G. hirsutum x aridum	L 13.3 : 1 R 29.7 : 1	20.2 : 1
6x G. hirsutum and G. barbadense x thurberi	L 21.2 : 1 R 45.8 : 1	33.5 : 1
6x G. barbadense x gossypioides	L 55.1 : 1 R 79.1 : 1	61.2 : 1

Since the amount of segregation in a given hexaploid is a reflection of degree of non-preferential chromosome pairing, which in turn depends on chromosome homologies existing between the species brought together in the hybrid, this array of segregations represents the phylogenetic interrelationship of G. hirsutum and each species of the American diploid group.

As can be seen from a perusal of the first data column of the table, the segregation for leaf-shape is narrower than that for anthocyanin coloration in each of the hexaploids with the exception of the raimondii type (here the difference in segregation for L and R is not significant). The best explanation would seem to be that chromosome structural differentiation concommittant with speciation in this American diploid group has rearranged the chromosomes carrying the leaf-shape and anthocyanin loci (and probably all other chromosomes as well) to a similar degree

within each of the species. The chromosomal structural alteration which would then explain the difference in segregation between L. and R in these G. hirsutum x American hexaploids probably occurred in the New World amphidiploid subsequent to its origin. The assumption that chromosome structural differentiation has been roughly similar for all chromosomes of a given species can be tested by studying the segregation of synthetic amphidiploids of hybrids combining species of the Wild American group.

As yet meager and therefore preliminary data on multivalent frequency in these same hexaploid types indicates, as expected, a close correlation between segregation and multivalent frequency; hexaploids that give narrower segregation ratios show higher multivalent frequencies (i.e., 6x G. hirsutum x raimondii: seg. 9.3:1, mult. freq. 6.16 per cell) and hexaploids with wider segregations show lower multivalent frequencies (i.e., 6x G. barbadense x gossypoides: seg. 61.2:1, mult. freq. 1.25 per cell). Multivalent frequency in 6x G. hirsutum x arboreum and 6x G. barbadense x arboreum averages 8.25 multivalents per cell. As previously reported, segregation in these hexaploids is very close to the autoploid 5 : 1 ratio.

Segregation for most marker loci from synthetic amphidiploids of the two Asiatic diploids have clustered near the 5 : 1 ratio. The segregation for leaf-shape, however, in 4x G. arboreum x herbaceum is 8.98: 1. It is of interest that the leaf-shape locus is contained in a three-point linkage group in which there is a significant depression in crossing-over in the interspecific G. arboreum x herbaceum hybrid (c.o., 24.3%) as compared with the same linkage group in G. arboreum (c.o., 37.0%). Thus two independent tests indicate chromosome structural divergence, at least for the chromosome carrying leaf-shape, between G. arboreum and G. herbaceum.

NICOTIANA

1) Segregation in Synthetic Amphiploids.

The occurrence of unexpectedly low segregation ratios in certain synthetic amphiploids of this genus was the major finding of the year. In Gossypium amphiploids the lowest ratios obtained were approximately 5 : 1. With complete homology somewhat lower ratios with a minimum value of 3.7 : 1 could theoretically be obtained for distal loci on the chromosome ("chromatid segregation"). However, some of the Nicotiana ratios were significantly below 3.7 : 1. Our heuristic working hypothesis postulates that loss of chromosomes may be the cause. Indeed, it was found by Miss Yang that a proportion of the pollen grains produced by Nicotiana amphiploids carried fewer than the balanced number of 36 chromosomes. Chromosome elimination at meiosis was also evident from the frequent appearance of micronuclei in pollen quartets. The quantitative relationships are under study. A considerable body of data is also accumulating which indicates in polyploids of Nicotiana losses of chromosomes from somatic tissues. These result in sectoring of branches, and in distorted progeny ratios when premeiotic cells are affected. This again it seems to be more frequent in tobacco than in cotton material.

Apparently tobacco polyploids are either less sensitive to aneuploidy, or else more prone to losses of chromosomes from the division spindles and it is hoped that an explanation for the difference between the two genera can be formulated in the near future.

The following is a brief account of the segregational behavior of the various hexaploids synthesized from tobacco and its nearest relatives:

a) 6x N. tabacum x tomentosiformis: Hexaploid combinations of these two species gave very small segregation ratios ranging from 2.3 : 1 for the white corolla locus to 4.7 : 1 for yellow green, with fasciated, purple bud and yellow burley giving intermediate results. A typical 5 : 1 autoploypoid ratio was found in no case.

b) 6x N. tabacum x otophora: Consistently, the ratios were larger than in a) ranging from non-significantly below 5 : 1 to about 8 : 1 for the fasciated, purple bud, white seedling and yellow burley factors in that order. A considerably larger ratio for yellow green segregation was observed, namely 15 : 1. This large ratio may have its cause in the structural differentiation of the chromosomes carrying yg alleles, or it may merely be due to reduced viability of yg segregants. The yellow green plants were weak, as a rule, which favors the latter explanation.

c) 6x N. tabacum x setchellii: Only segregation for the white corolla locus has been obtained thus far which gave a segregation of 493: 71, or 7 : 1, which is highly significantly larger than the autotetraploid ratio.

The three amphiploids listed under a) to c) are of particular interest in connection with the problem as to which member of the Tomentosa section is closest to the parental type of N. tabacum. N. tomentosiformis, N. otophora and N. setchellii belong to that section. Our data favor N. tomentosiformis somewhat, but they also point to the difficulties encountered when the method of measuring taxonomic relationships by means of amphiploid segregation frequencies is applied to a genus with frequent aneuploidy. Since no ratios as large as those in some of the Gossypium hexaploids were found the results also indicate that far less chromosomal differentiation has occurred among the species of the Tomentosae than among the D-species of Gossypium.

d) 6x N. tabacum x sylvestris: N. sylvestris represents the second genome of the natural amphiploid which is N. tabacum. Two of the markers in the hexaploid combinations gave wide ratios, as reported previously: yellow burley gave about 12 : 1 and yellow green 26 : 1. The remaining loci, however, segregated with ratios at approximately, or slightly above the 5 : 1 level of autopolyploidy. Five or six loci gave such ratios : carmine, ruffled, spontaneous necrosis, white seedling, Lumbreras syndrome (s. below) and possibly petioled. (The petioled case is

of interest since it has not been possible to locate the Pt gene on a particular chromosome by the monosomic method (work of the California group) .It was thought that at least the subgenome could be determined by the amphiploid segregation method. And indeed, the segregation observed suggests location in the sylvestris subgenome, but since the phenotypic expression is not clearcut the result should be accepted with caution. The test for the other genome still needs to be made.]

N. tabacum x sylvestris hexaploids are also suited to test the possibility of an influence of the cytoplasm on chromosomal behavior in polyploids. This is possible since reciprocal hybrids can be made between N. tabacum and N. sylvestris. The preliminary results indicate that the cytoplasm has no marked influence on the segregation ratio:

Locus	Hexaploid segregation ratio In tabacum cytoplasm	In sylvestris cytoplasm
yb	11.6 : 1	14.3 : 1
pk	4.2 : 1	3.6 : 1

2) A Case of Introgression.

Some twenty years ago a chromosome from the more distant species N. glutinosa was introduced into tobacco via a hexaploid in order to provide resistance to the mosaic disease. This chromosome characteristically fails to pair with its tabacum homologue. Intensive backcrossing of the substitution type with selection for agronomic desiderata has resulted in 24 II tobaccos with resistance. Resistance now is carried undoubtedly in a chromosome consisting largely of tabacum chromatin, since 24 II are generally formed in heterozygotes. It was of interest to find out how much of the glutinosa segment had been retained by studying the degree of association of the rebuilt chromosome with an intact glutinosa chromosome. This was done by means of an analysis of hybrids between a modern agronomic line of mosaic resistant tobacco with Holmes Samsoun, the original substitution strain

which contained an intact pair of glutinosa chromosomes plus 23 II's of tobacco chromosomes. The result indicated that an appreciable glutinosa segment was still carried in the agronomic variety. The evidence consisted in a) a proportion of cells with 24 II, roughly 1/5 of the total and b) a high tendency of the unpaired chromosomes to lie side by side on the metaphase plate in those cells which had 23 II + 2 I in a sort of "secondary association". Some tendency for "secondary association", though of lesser extent, was discovered, surprisingly, also in the controls containing the intact glutinosa chromosome and its pure tabacum partner.

3) New Genetic Markers

Two mutants which were in their expression like others already available were found by the test of complementarity to be non-allelic with the older markers.

a) A white flowered type recently introduced by Dr. Rick from Peru gave colored offspring when crossed with wh.

b) Japanese "aurea" which looks like yellow burley gave normal green from aurea x yellow burley.

Two virescent types one of which has normal flowers while the other has reduced and otherwise abnormal flowers were found to be allelic since their F_1 was virescent. These two mutants had been obtained from the X-ray work of Dr. L. Apple.

Another X-ray product with chlorophyll variegation was found to be heritable and a rugose leaf type obtained from the breeding plots of Mr. O. Neas turned out to be dominant.

One often encounters in interspecific hybridization that the combination of particular strains of two species results in weak, sometimes "semi-lethal"

hybrids whereas usually the cross of the same species results in vigorous progeny. One such case was encountered in N. tabacum x sylvestris when the "Lumbreras" race of the latter species was used. The hybrid, while viable and capable of flowering, is weak with necrosis on the lower stem and has small and very brittle leaves. The segregation platter of the "Lumbreras syndrome" in the amphiploid 6x N. tabacum x sylvestris Lumbreras suggests that a single factor from N. tabacum is complementary with an as yet unknown number of factors from N. sylvestris.

The further inheritance of these various mutants will be investigated before their employment as markers in the interspecific program.

PUBLICATIONS SINCE LAST ANNUAL REPORT:

Gerstel, D. U. and L. L. Phillips. Segregation of synthetic amphiploids in Gossypium and Nicotiana. Cold Spring Harbor Symposia of Quantitative Biology 23: 225-237. 1958.

Gerstel, D. U. Segregation in raw allopolyploids in Nicotiana. Genetics 44: 513. 1959.

Phillips, L. L. and D. U. Gerstel. Segregation in new allopolyploids of Gossypium. III. Leaf-shape segregation in hexaploid hybrids of New World cottons. J. Heredity 50: 103-108. 1959.

Sarvella, P. A. Multivalent formation and genetic segregation in some allopolyploid Gossypium hybrids. Genetics 43: 602-619. 1958.

RESEARCH PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

IN APPLICATION FOR A RESEARCH GRANT

1. Name and address of institution:

North Carolina State College,
Raleigh, North Carolina

2. Principal Investigator:

Dr. D. U. Gerstel

3. Title of proposed research:

Studies on the segregation in artificial amphidiploids in the genus Gossypium.

4. Description of proposed research:

A. Background

Amphidiploid and similar polyploid hybrids have attracted great attention because of their importance in plant evolution (s. Stebbins, G. L., 1950) and as tools in crop breeding (for examples see papers by McFadden and Sears, 1947, on wheat, Harland, 1941, and Beasley, 1942, on cotton, and Gerstel, 1948, on tobacco). Despite their importance, the genetic behavior of newly synthesized amphidiploids has only rarely been investigated. Best known are those examples which show little segregation but instead preferential pairing between the chromosomes of each parent species (as Nicotiana digluta, Clausen and Goodspeed, 1925) or random pairing and tetrasomic segregation as in Fragaria bracteata x vesca (Yarnell, 1931). However, such clearcut behavior is not found always, as already the classical example of Primula Kewensis indicated, where some segregation was observed and attributed to the occurrence of quadrivalent chromosomes in that amphidiploid (Newton and Pellew, 1929). Another example is the case of Rubus, described by Crane and Darlington (1929) where one taxonomic character segregated in the amphidiploid R. rusticanus x thyrsiger, but another did not.

These studies are rather old, but interest in the problem seems to be revived quite recently; Stebbins and Grant both reported at the 1952 meetings of the Genetics Society (s. Records) on taxonomic studies employing the segregation of raw amphidiploids as a tool. The potato group at the University of Wisconsin has started to attack the problem in Solanum amphidiploids (unpublished).

In Gossypium are available a number of species with various degrees of taxonomic relationships. Their F_1 hybrids may exhibit complete pairing of chromosomes at meiosis or fail to pair altogether; several intermediate stages are known (extensively studied by Skovsted, Beasley, Brown, Gerstel and others between 1930 and the present). A fairly large number of excellent marker genes is known in a number of these species (Harland, 1939, and Hutchinson and Silow, 1939) and are available in the cotton collection at

N. C. State College. Also available, or in the process of being synthesized are a selected number of amphidiploids between the various species containing these genetic markers.

The main object of the proposed investigations is to study and compare the genetic behavior of these amphidiploid stocks and beyond this to analyze the barriers against recombination and gene introgression in the genus Gossypium.

B. Recent work by principal investigator.

a. Cytological investigations.

Comparative cytological studies on various species and their F_1 hybrids revealed that the two cultivated species of Asia (G. arboreum and G. herbaceum) differed in one major chromosomal feature, a reciprocal translocation. Following this translocation through the various species of the genus it was discovered that G. herbaceum resembled the ancestral, primitive G. anomalum and also the recent New World cotton more closely than did G. arboreum. Thus it appears not unlikely that G. herbaceum is an important link in the evolutionary sequence while G.arboreum is merely a side-branch. This is of interest as for geographical reasons G. arboreum had been regarded in recent years the closer relative of American cultivated cotton and was the only one considered in breeding studies concerned with gene transfer.

b. Genetical studies.

To our knowledge of mechanisms which prevent hybridization between species of Gossypium a case has been added of gene induced lethality of interspecific hybrids. The sanguineum race of G. arboreum and Upland carry certain complementary genes which cause their hybrid to die when coming into flowering or somewhat earlier, depending on the specific genotypes and on environmental conditions. When kept at temperatures which are slightly below the optimum for cotton the lethal plants can survive. The lethal genes are proving valuable markers for the amphidiploid studies.

c. Segregation in artificial polyploids.

The similarity of chromosomes of two species cannot only be investigated by studying their pairing behavior in the F_1 hybrid but may be analyzed genetically with greater precision in their amphidiploid hybrid. Such amphidiploid hybrids, which are obtained by colchicine treatment from the F_1 hybrids, possess four instead of the usual two chromosomes of each kind, i.e. two from either species under investigation. If similar, all four chromosomes associate at random; if dissimilar parental chromosomes tend to associate more often and the type of association can be told from the genetical composition of the progeny.

Using this technique it became apparent that (apart from the reciprocal translocation mentioned above) chromosomes of the two Asiatic species were similar as tetrasomic ratios were obtained (for

three out of 13 chromosomes tested) but greatly dissimilar from those of wild G. anomalum as segregants were rare (five chromosomes tested). New World cotton chromosomes showed a considerable amount of association with wild American G. raimondii (the gametic ratio was of the magnitude 1 AA : 14 Aa : 1 aa) but considerably less with the chromosomes of G. thurberi, another wild American species (approximately 1 : 40 : 1). Two out of 13 chromosomes were tested for each of the latter two amphidiploids and several hundred plants were grown in each case to assure significance of differences.

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5. Procedure

a. Genetical analyses

These limited results indicate enormous differences in the frequencies with which recombination takes place in different allopolyploids. It will be very desirable to expand these results and particularly to test to what extent a particular polyploid hybrid shows uniform behavior, or whether different markers in the same hybrid give different segregation ratios. Furthermore, the influence of various stocks from the same species upon segregation of amphidiploids remains to be tested.

In addition to those allopolyploids now under study a number of new marker lines of G. arboreum and of cultivated New World cottons have been crossed with wild species. The most important of these are three New World marker lines containing eight heretofore untested alleles which have been mated with both G. raimondii and G. thurberi. These hybrids, and others involving Asiatic species and G. anomalum, will be treated with colchicine to produce fertile polyploids which in turn will be crossed back to recessive New World lines for testing.

The resulting aneuploids will be backcrossed to the recessive testers to obtain additional evidence on the behavior of the introduced chromosomes.

b. Cytological studies.

Cytogenetic analyses are needed to decide whether widely differing segregation ratios found in synthetic polyploids can be attributed to variation in meiotic behavior or to other alternatives Among these may be mentioned differential gamete and zygote elimination.

Available in the greenhouse are a number of synthetic allopolyploids of various degrees of taxonomic relationship between the

component species (4n G. arboreum-herbaceum, 4n G. arboreum-anomalum, 4n G. herbaceum-anomalum, 4n G. arboreum-thurberi and 6n New World-wild American combinations).

These materials are to be subjected to quantitative cytological analysis to ascertain a) whether meiotic behavior is essentially the same for polyploids synthesized from different lines of the same species and b) whether correlations exist between meiotic behavior and genetic segregation. Special attention will be paid to the number of multivalents and univalents. These cytological experiments are intended to run simultaneously and on the same plants as the segregation studies outlined above.

c. Embryology of non-functional Gossypium hybrids.

It is desirable to study the ways and means by which gametes and zygotes may become aborted in these materials for its bearing on differences in genetic ratios. A short study by Beasley (1940) indicated that in certain Gossypium hybrids embryo abortion probably followed the pattern frequently encountered in the Angiosperms as described in a series of studies by Brink and Cooper (s. review of 1947). Beasley made no detailed study at the time and many more hybrid and polyploid combinations have become available since. In our materials certain patterns have become apparent in the abortion features of hybrids between species of different chromosome numbers. Thus in 2n x 4n and 4n x 6n matings seeds were produced which were regularly of normal size by contained a shrivelled embryo and no endosperm. In the reciprocal crosses only a few ovules developed at all, but these reached full maturity (in 6n x 4n) or attained a stage at which they could be reared on an artificial medium (in 4n x 2n). No detailed studies have as yet been undertaken.

The artificial hexaploids and artificial and natural tetraploids mentioned above may be utilized for a comparison of normal development resulting from selfing and in pure species with the abnormal ones produced in the various cross combinations. In addition to the reciprocal crosses between plants of different degrees of ploidy it is desirable to study abortion features in cases where ploidy does not have a role, as, e.g., in matings between certain lines of the diploid species G. arboreum and G. herbaceum, and between G. sturtii, G. davidsonii and other wild American species.

6. Facilities:

Land and field labor are available near the campus and at the McCullers Experiment Station nearby. Adequate greenhouse space and air conditioned cytological laboratories with necessary equipment are provided in Williams Hall.

As an advantage for the work at State College may be mentioned the fact that an active group is engaged in cooperative studies of various aspects of cotton breeding methodology and cotton genetics in the Department of Agronomy, Genetics Faculty and in the Institute of Statistics.

7. Personnel:

Dan Ulrich Gerstel
Born October 23, 1914 at Berlin-Dahlem, Germany.
Received secondary education at a Berlin Gymnasium.
Attended Universities of Grenoble, France; and Fribourg, Switzerland.
Received B.S.,M.S. and Ph.D. (1945) at University of California at Berkley, where he also served as Teaching fellow in Botany and Associate in Genetics.
Associate Geneticist on Guayule Project for U.S.D.A. from 1947-50.
Assistant Professor at N. C. State College from April 1950 to date.
Member of American Society of Agronomy, Genetics Society, Evolution Society, American Society for the Advancement of Science, and Sigma Xi.

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8. Budget:

	First Year		Second Year		Third Year	
a/ Salaries	State	NSF	State	NSF	State	NSF
Prin. Invest. D. U. Gerstel	-	-	-	-	-	-
2 Grad. Students		$3600		$3600		$3600
b/ Permanent Equipment						
Misc. Equipment	$200	200	$200	-	$200	-
c/ Expendable Equipment and Supplies	$500	500	500	500	500	500
d/ Travel	300	100	300	100	300	100
e/ Other Direct Costs						
Contractual including photography	100	100	100	100	100	100
		4500		4300		4300

Total requested for three year period $13,100

9. Approval:

D. U. Gerstel,
Principal Investigator

W. E. Colwell, Head
Department of Agronomy

R. V. Cummings,
Director of Research
Agricultural Experiment Station

May, 1953