GENETIC STUDIES OF AERIALOGENESIS IN *NEUROSPORA CRASSA*

GENETIC STUDIES OF SOME SPONTANEOUSLY ARISING DIFFERENCES IN AERIALOGENESIS BETWEEN *NEUROSPORA CRASSA* STRAINS.

By

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To Dr. Claude W. Hinton; without his initial inspiration, I would probably have never attained this goal.

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ABSTRACT

The purpose of this study has been to genetically analyze the aerialogenesis steps during differentiation of the fungus, *Neurospora crassa*. A new technique was derived for collecting data relating to the extension growth of aerial hyphae. This study comprised over 325,000 observations of aerial extension growth of over 46,000 colonies. These data were then analyzed by several new methods to characterize the aerial growth of the strains involved on medium containing 1% sorbose. Due to the large number of observations, a computer analysis procedure was utilized with the data. Once the phenotypes had been quantitatively described, hypothesized genotypes and the variant alleles segregating could be accurately followed.

At least four genetic loci were observed to affect aerialogenesis in the presence of sorbose. Three of these loci were present, in two allelic forms each, in the original strains. A variant allele at a fourth locus arose spontaneously during the course of this study. Several allele interactions have been characterized and some information is presented on gene locations.

The question of the relationship between aerialogenesis and conidiogenesis was also considered.

No effects were seen on the qualitative aspects of conidiogenesis (macro or microconidiation). However, in several asci, distinct quantitative effects on conidiogenesis were observed segregating as expected of nuclear genes. In these asci the quantitative changes in conidiogenesis were not correlated to quantitative changes in aerialogenesis. Hence, it appears that these two important developmental events can be separated genetically.

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INTRODUCTION

Much remains to be learned about the mechanisms controlling growth and differentiation in organisms. The relatively simple form of the eukaryote, *Neurospora crassa*, and the ease with which its environment may be manipulated facilitate the study of development in this organism. A great number of progeny may be produced asexually as conidia or crosses may be made between specific strains and the meiotic products gathered, randomly or in order, for further tests. Previous studies have shown that asexual development of *N. crassa* on solid media includes the differentiation of laterally growing vegetative hyphae into aerially growing hyphae (aerialogenesis) (Siegel et al., 1968). This process is often followed by the differentiation of conidia on the aerial hyphae, with the aerial hyphae then becoming known as conidiophores. Only multinucleate macroconidia are thought to be produced by the process of aerial hyphae differentiating into conidiophores, with conidia forming on the tips of the hyphae (Grigg, 1960a; Siegel et al., 1968). Uninucleate microconidia are produced as clusters along the mycelia near the surface of the colony (Grigg, 1960a).

Prior to the initiation of my study, very little had been published on the genetics of aerialogenesis or conidiogenesis. Siegel et al. (1968) had presented a preliminary report of a technique for temporally separating these processes. However, the problem of aerial extension growth had not been attacked as a means of quantitating the genetic aspects of aerialogenesis. Early in the course of my study a report of protein changes during synchronous conidiation, using the technique of Siegel et al. (1968), was published from Siegel's lab (Urey, 1971). The study concentrated on the conidiation step of the asexual reproductive process although some attempt was made to quantitate the production of aerial hyphae. It was felt that use of this or a similar technique for quantitation of aerialogenesis would be of insufficient sensitivity for defining different genotypes affecting the aerialogenesis process. More recently, Siegel's group has published several papers on mutants affecting the macroconidiation process (Selitrennikoff et al., 1974; Matsuyama et al., 1974; Nelson et al., 1975 a and b, 1976). These were isolated using mutagenized strains and a selective procedure utilizing their original technique for inducing synchronous conidiation (Siegel et al., 1968). This technique would appear best adapted to conidiation studies.

Grigg (1958), 1960 a and b) has reported on the genetic control of conidiation using the peach microconidial (*pc*^{m}) mutant (Barratt and Garnjobst, 1949). He refers to this mutant as *m* in his reports where he has shown the interactions of various loci in the conidiation and aerialogenesis processes, although he was unable to quantitate the latter processes. The wild type strain produced less than 1% microconidia and Grigg (1958) states that in order to significantly increase the percentage of microconidia there must be a macroconidiogenesis suppressor present. Any qualitative affects on conidiogenesis may also affect aerialogenesis or may be brought about by affects on the aerialogenesis step in the differentiation process.

Although specific work on the quantitative aspects of aerialogenesis is limited, numerous other morphologically distinct *Neurospora crassa* strains have been isolated and more than one hundred loci implicated in their genetic control (Garnjobst and Tatum, 1967; Murray and Srb, 1962; Mishra, 1976). Garnjobst and Tatum (1967) classified ninety known morphological strains into six phenotypic classes; true colonials *(col)*, spreading colonials *(speo)*, semi-colonials *(smco)*, miscellaneous spreading morphological types distinct from wild type *(mo)*, another miscellaneous type with distinct nonspreading

mycelial growth, and environmentally influenced morphologicals *(moe)*. Strains which produce aerial hyphae under known genetic control were not specifically included in their study. The strains used in this study, with altered aerialogenesis, would probably fit into the *moe* class of mutants due to the influences of environment on their morphology.

Previous studies of other morphological types in *Neurospora crassa* have confronted the problem of development using predominantly one of three distinct methodologies. Several good review articles are available which summarize these methodologies and the results obtained (Bartnicki-Garcia, 1973; Brody, 1973; Mishra, 1976; Nelson, et al., 1975b; and Turian, 1966). One method of attacking the problem involves biochemical studies of different fungal structures to define the chemical differences between mutant and wildtype strains under various environmental conditions (Mahadevan and Tatum, 1965; Bartnicki-Garcia, 1973). A second method involves analysis of strains with their morphology affected by a single locus, as determined in genetic tests, and determination of the specific metabolic lesion associated with mutation at that locus (Brody, 1973; Mishra, 1976). The third technique employs light and electron microscopy of surface and ultrastructural alterations of strains grown under various environmental conditions (Turian, 1966;

Bartnicki-Garcia, 1973).

Alterations in the chemical composition of the rigid cell wall of *Neurospora crassa* were implicated early as affecting morphogenesis (DeTerra and Tatum, 1961 and 1963), although not necessarily aerialogenesis. These authors took advantage of the known morphological affects of sorbose and snail gut digestive enzyme in inducing colonial growth. Sorbose produces its colonializing effect as a relatively non-utilizable carbon source while snail gut enzyme treatment releases large quantities of glucose from cell wall polymers. DeTerra and Tatum found variation in cell wall constituents between colonies grown on media containing either or neither of these paramorphogenetic substances. Mahadevan and Tatum (1965, 1967) used sorbose induction of colonial growth as well as colonial mutants in their studies of variation in the structural polymers of the *Neurospora* cell wall. Mahadevan and Tatum separated the wall into four consistantly recognizable fractions. Fraction I is soluble in alkali and contains complexed peptide and polysaccharide (glucan) including galactosamine, glucose, and glucuronic acid. Fraction II is also a glucan fraction but is alkali-insoluble and acid soluble. Fraction III is easily digestible by laminarinase and contains glucan in a β-1,3 linkage. The fourth Fraction is glucosamine (chitin). One or more of these polymers was found to be consistently altered in single locus

colonial growth mutants and in sorbose induced colonial growth. The level of Fraction I in relation to the rest of the cell wall polymers appeared most important in correlating gene or chemically induced morphological changes. In each case studied, alteration in the morphology of the fungus was associated with alteration in cell wall constituents, whether the alteration was caused genetically or by paramorphogenetic chemicals added to the media. In no cases, were strains selected for altered aerialogenesis subsequently analyzed chemically for cell wall composition. However, when considering the varying morphological types analyzed, it is quite reasonable to expect strains with altered patterns of aerialogenesis to have the composition of their cell walls altered. Conversely, changes in levels of cell wall polymers may have a recognizable affect on aerialogenesis if aerial hyphae production is studied in a quantitative way.

Once the composition of the cell wall had been correlated with morphological alterations, several investigators began to study known morphological mutants, looking for the primary enzymatic defects. Several morphological mutants were found to have defects in enzymes thought to be involved in the synthesis of cell wall precursors, in enzymes affecting coenzyme metabolism, in

enzymes affecting metabolism of small potential regulatory molecules, or in enzymes capable of degrading specific cell wall constituents (Table 1). Three separate loci (*col-2, bal* and *fr*) associated with morphological changes were also implicated in alterations of glucose-6-phosphate dehydrogenase activity by Brody and Tatum (1966) and Scott and Tatum (1970). Two phosphoglucomutase activities are known in *Neurospora* and were shown to be affected by 2 separate loci (Brody and Tatum, 1967; Mishra and Tatum, 1970). Two loci were also implicated in the production of 6-phosphogluconate dehydrogenase activity by Abramsky et al., (1971) and Scott and Abramsky (1973). Pina and Tatum (1967) showed that a mutant of the *inos* locus on LG V reduced the activity of a glucocycloaldolase. Glucoseisomerase and glucoamylase were affected by alterations of specific loci (Murayama and Ishikawa, 1973, 1975). Terenzi et al. (1974) showed a correlation between mutation at the crisp-1 *(cr-1)* locus and adenyl cyclase activity, and later (1976) found more specific affects of cAMP on morphology and on the change from lateral vegetative hyphae to aerial growth and conidiation. At least two loci were found to control cell wall autolysing activity by Selitrennikoff et al. (1974). More recently this same group has found a locus *(nada)* on LG IV associated with

TABLE 1

A Summary of Morphological Mutants Referred to, Including the Linkage Group and Biochemical Defect, Where Known.

STRAIN (LG)	BIOCHEMICAL DEFECT	PHENOTYPE	REFERENCE
pat (I)	-	resistance to paramorphogenetic affect of sorbose	Misnra and Tatum (1972)
fr (I)	glucose-6-phosphate dehydrogenase	nonconidial, delicate branching	Scott and Tatum (1970)
rg-1 (I) (*col-7* allelic)**	phosphoglucomutase I	poorly conidiating colonial growth	Brody and Tatum (1967) Mishra and Tatum (1970)
rg-2 (I)	phosphoglucomutase II	poorly conidiating colonial growth	Brody and Tatum (1967) Mishra and Tatum (1970)
T-9 (I)	glucoamylase	sorbose resistant, poor growth	Murayama and Ishikawa, (1973, 1975)

TABLE 1 (continued)

STRAIN (LG)	BIOCHEMICAL DEFECT	PHENOTYPE	REFERENCE
cr-1 (I)	adenyl cyclase	early uniform conidiation on a slant	Terenzi et al. (1974)
csp-1 (I)	cell wall autolysing enzyme(s)	altered conidial separation	Selitrennikoff et al. (1974)
pe^{m} (II)*	-	microconidiating no long aerial hyphae	Barratt and Garnjobst, (1949)
bal (II)	glucose-6-phosphate dehydrogenase	restricted hemispherical colony	Scott and Tatum (1970)
col-10 (II)	6-phosphogluconate dehydrogenase	colonial growth	Abramsky et al. (1971); Scott and Abramsky (1973)
sor^{r}*-C* (III)	regulatory locus?	resistance to paramorphogenetic affect of sorbose	Klingmuller et al. (1970)
col-1 (IV)	-	colonial growth	Grigg (1960a and b)
gpi-1 (IV)	glucoseisomerase	no growth on fructose, colonial on glucose	Murayama and Ishikawa, (1973, 1975)

TABLE 1 (continued)

STRAIN (LG)	BIOCHEMICAL DEFECT	PHENOTYPE	REFERENCE
gpi-2 (IV)	glucoseisomerase	no growth on fructose, colonial on glucose	Murayama and Ishikawa, (1973, 1975)
nada (IV)	NAD(P)ase deficiency	altered macroconidiation	Nelson et al. (1975a)
gal-1 (IV)	galactokinase	restricted growth or none on galactose medium	Mishra (1976) citing Rand (1975)
gal-2 (IV)	UDPG pyrophosphorylase	restricted growth or none on galactose medium	Mishra (1976) citing Rand (1975)
bd (IV)	-	circadian rhythm of conidiation and aerial growth; also other possible alleles with altered period length	Sargent et al. (1966) Sargent and Woodward (1969) Feldman and Hoyle (1973)
inos (V)	glucocycloaldolase	inositol auxotroph	Pina and Tatum (1967)
pk-2 (V)$^{++}$	L-glutamine-D-fructose-6-phosphate amidotransferase	8 unordered spores in a balloon shaped ascus	Russell and Srb (1974) Murray and Srb (1962)

TABLE 1 (continued)

STRAIN (LG)	BIOCHEMICAL DEFECT	PHENOTYPE	REFERENCE
cl (V)$^{++}$	if allelic to *pk-2*, then same Biochemical defect	noncircadian rhythmic aerialogenesis	Berliner and Neurath (1966)
sor^{r}*-A* (VI)	transport enzymes?	resistance to paramorphogenetic affect of sorbose	Klingmuller et al. (1970)
sor^{r}*-B'* (VII)	transport enzymes?	resistance to paramorphogenetic affect of sorbose	Klingmuller et al. (1970)
col-2 (VII)	glucose-6-phosphate dehydrogenase	colonial growth	Brody and Tatum (1966)
col-3 (VII)	6-phosphogluconate dehydrogenase	colonial growth	Abramsky et al. (1971); Scott and Abramsky (1973)
csp-2 (VII)	cell wall autolysing enzyme(s)	altered conidial separation	Selitrennikoff et al. (1974)
su^{m} (-)	-	suppresses phenotypic affect of *pe*^{m}	Grigg (1960a and b)
acon^{t} (-)	-	aconidiate	Grigg (1960a and b)

TABLE 1 (continued)

STRAIN (LG)	BIOCHEMICAL DEFECT	PHENOTYPE	REFERENCE
spco-1 (-)	cell wall autolysing enzyme	spreading colonial	Mahadevan and Mahadkar (1970)

++ *pk* and *cl* are allelic (Mishra, 1976)
* p^m referred to as *m* by Grigg, 1960a and b, and more recently as *pe* by Barratt and Ogata (1975)
- if unknown LG, or biochemical defect
** Mishra (1976)
other references used in construction of this table: Barratt and Radford (1969, 1970); Barratt and Ogata (1970, 1975) and Mishra (1976)

changes in macroconidiation and causing deficiencies in NADase and NADPase (Nelson, et al., 1975a). However, Nelson et al. (1976) pointed out that although the NAD(P)ase appeared to be developmentally regulated, the appearance of the enzyme need not be implicated in the regulation of development. They found that NAD(P)ase levels appeared to be closely regulated throughout the development of wild type *N. crassa*, with a distinct accumulation of the enzyme activity during aerialogenesis. However, by using mutant strains, they also showed that the accumulation of catalytic activity of this enzyme was not required for the differentiation of aerial hyphae or viable conidia. Hence, the conclusion that NAD(P)ase is developmentally regulated but not germinal to the regulation of development. Previously, Mahadevan and Mahadkar (1970) had also found a locus affecting cell wall autolysing activity, although results of allelism tests with the two loci described by Selitrennikoff et al. (1974) have not been published. The results of tests with snail gut digestive enzyme had already shown that partial degradation of the cell wall affected morphology (DeTerra and Tatum, 1961). *Neurospora* can produce extracellular enzymes which can partially degrade its cell wall, and hence, alter branching patterns and morphology in a similar fashion to the snail enzyme. The observed correlation between changes in autolysing

activity and morphological changes is consistent with these various lines of evidence. Russel and Srb (1974) have shown the peak-2 *(pk-2)* locus to be involved in L-glutamine-D-fructose-6-phosphate amidotransferase activity. Rand (1975), as reported by Mishra (1976) has also shown a locus involved in galactokinase activity and another in UDPG pyrophosphorylase activity. Wrathall and Tatum (1973, 1974) analyzed the peptides found in the cell walls of several strains of *Neurospora crassa*. These wall peptides were shown to comprise at least 8.63-9.35% of the cell wall from wild type strains. They divided these peptides into two classes; 1) those not associated with wall structure but in transit or trapped in the wall and 2) those hypothesized to be participating in the determination of morphology. Some twenty-three colonial strains were deficient in wall peptides, having a peptide fraction of only 3.34-6.64% of the cell wall.

A complete unified picture of the steps in cell wall biosynthesis and the controlling genetic loci is not yet available. However, the numerous examples of related genetic mutation, alteration of specific enzyme activity, alteration in cell wall constituents and change in morphology all point to an *in vivo* relationship between these observations.

My observations relating two of these variables, a gene locus and morphological change, in the case of aerialogenesis, indicates that further work would probably verify alterations present in the other variables, enzymes and cell wall constituents.

Microscopic studies of fungal growth and conidial differentiation have been recently reviewed by Bartnicki-Garcia (1973). Microscopic studies by Weiss and Turian (1966) and Turian and Matakian (1966) showed effects of chemical alterations of the medium on conidiation, describing macroconidia formation as a process of constriction and severance of segments of preformed aerial hyphae. Rizvi and Robertson (1965) noted the disintegration of the hyphal apices of colonies grown on sorbose and not when grown on medium containing snail gut digestive enzyme, even though both cause a colonial morphology. Later, the same authors (Robertson and Rizvi, 1968) described water relations in *Neurospora crassa* hyphae which indicated a distinct turgor pressure at the hyphal apex essential for tip elongation. Mishra (1976) reported distinct differences between micro and macroconidial exterior surfaces using the scanning electron microscope and indicated the macroconidia were more similar to vegetative hyphae in surface appearance. A *rg* mutant and several osmotic mutants showed differences from wild type in their surface architecture using similar

techniques. Several studies from Turian's laboratory, including Turian et al., 1973; Rossier et al., 1973 and Dicker et al., 1975, have attempted to describe the ultrastructure of the conidiation process. Trinci's group (Trinci, 1974; Trinci and Collinge, 1973b, 1975) has studied the ultrastructure of hyphal extension including branch initiation and septal plugging. Koch (1975) presents a more theoretical model of mycelial growth in two and three dimensions indicating that the two forms of growth are different. In two dimensional growth (i.e. across an agar surface) the amount of mycelium invading a new space is small in relation to the final amount of mycelium that space can support. In three dimensional growth (i.e. in liquid culture) the invading mycelium is almost equal in amount to the final carrying capacity of the invaded space. Koch's model did not relate aerialogenesis and colony diameter to three dimensional growth. Considering the genetic and environmentally caused variation in these processes it might be very difficult, if not impossible, to create a three dimensional model using the criteria of colony diameter and aerial growth. The amount of aerial hyphae extending from the colony is highly variable and appears independent of colony diameter.

Another means of attacking the problem of conidiogenesis and aerial hyphae production in *Neurospora*

approach involves the use of a strain showing a marked circadian rhythm of aerialogenesis and conidiation. One of these strains, designated *bd* (band) and first described by Sargent et al. (1966) and more fully by Sargent and Woodward (1969), produces a characteristically circadian rhythm of aerial growth and conidiation. It was hoped that biochemical analysis of the band (aerial hyphae forming) and interband (nonaerial hyphae forming) regions could provide valuable insight into the events leading to aerialogenesis and conidiation. Correlations were found between banding patterns and (i) NADPH levels (Brody, 1970; Brody and Harris, 1973), (ii) the activity of 6 enzymes involved in glycolysis and the hexose monophosphate shunt (Hockberg and Sargent, 1974), (iii) RNA and DNA content and H^3-uridine incorporation (Martens and Sargent, 1974), (iv) AMP levels (Delmer and Brody, 1975), and (v) CO_2 production (Woodward and Sargent unpublished cited in Martens and Sargent, 1974). However, altered NADPH levels can affect several reactions and can be brought about by others (Lehninger, chap. 13, 1970). Hockberg and Sargent (1974) also found seven other enzymes which showed no oscillations in activity correlated with the visible circadian oscillations seen in the *bd* strain. Rhythms of circadian periodicity were found for RNA and DNA content and H^3-uridine incorporation in wild type and patch mutants,

which conidiate either continually or not at all under the experimental conditions (Martens and Sargent, 1974). No correlation was found between galactosamine or glucosamine levels and conidiation by Schmit and Brody (1975) even though both polymers have been correlated with cell wall changes during morphogenesis (Mahadevan and Tatum, 1965, 1967). Delmer and Brody (1975) found that there were no apparent oscillations in ADP or ATP levels even though AMP levels oscillated and suggested a partial uncoupling of mitochondrial oxidative phosphorylation which would agree with observations of a higher redox ratio and CO_2 production just after conidiation begins. The effect of altered CO_2 level agrees well with the work of West (1975) and others who have found correlations of CO_2 levels with conidiation, including induction of circadian conidiation bands in a wild type strain in a very low CO_2 environment. However, when these results are considered as a group there are no clear cut distinctions between the type of enzyme or parameter that has been observed to vary with a circadian oscillation, when compared to those which show no circadian oscillation. Thus, these studies have not, as yet, provided definitive answers to the question of what metabolic events are required for aerialogenesis or conidiation, although they may well provide such answers in the near future.

Bianchi (1964) worked with a strain which showed a rhythmic mycelial branching pattern and conidiogenesis, although not a truly circadian rhythm based on the ease with which it could be disrupted. His strain produced no growth on sorbose and he observed greatly enhanced aerial growth on glucose medium as temperature increased from 15 to 35°C. A heat sensitive factor was isolated from the glucose medium which eliminated the aerialogenesis rhythm. He suggested production of an aerial inhibitor which vegetative lateral hyphae must outgrow before more aerial hyphae might be produced. More recently, Halaban (1975) isolated a new mutant from a patch *(pat)* strain which showed periodic production of sparse and dense aerial hyphae and the inability to use carbohydrates. This mutant did not grow on minimal medium supplemented with carbohydrates, but did grow on acetate minimal medium. On acetate-glucose minimal medium it produced final yields similar to wild type or the original *pat* strain. The mutant appeared to be distal on the left arm of LG IV and allelic to *bd*, although phenotypically with a generally longer and more variable period length. The production of aerial hyphae in this mutant, similar to Bianchi's (1964), was not circadian according to its dependence on medium composition, high Q_{10} and insensitivity to light-dark changes. However, the conidiation rhythm appeared to be maintained throughout

the environmental changes which altered the aerialogenesis rhythm. Thus, Halaban (1975) suggested two separate rhythms; one circadian rhythm of conidiation as found in *bd* and *fst*, and a non-circadian rhythm of aerialogenesis similar to clock *(cl)* (Berliner and Neurath, 1965, 1966) and the mutant described in her report. This is one of the first reports of distinctly different cellular controls over aerialogenesis and conidiogenesis. Three other mutants, isolated from a *bd* strain by Feldman and Hoyle (1973), are closely linked, possibly allelic, and affect the period length specifically; two giving a shorter period and one a longer period. Mutants such as these may also prove useful in differentiating between controls on aerialogenesis and conidiogenesis by allowing analysis of both events while the strain is under an altered cyclic control.

L-sorbose is known to affect the morphology of wild type *Neurospora crassa* strains, causing them to grow colonially on agar media (Tatum et al., 1949). Since this discovery, sorbose-containing media have been used extensively to facilitate the testing of numerous strains on one plate. After greater than 100 hrs of adaptation to sorbose, on medium containing 0.2% glucose, which is depleted within about 30 hrs, sorbose appears to become metabolized via sorbitol, although colonial growth is

still maintained (Crocken and Tatum. 1968). Crocken and Tatum (1968) also state that along with the colonial growth, sorbose causes a failure to conidiate and a loss of up to 60% of the dry weight of a wild type culture, after the depletion of the small amount of glucose in the medium. The glucose initially present in the medium is able to inhibit sorbose uptake, even against a large concentration gradient, suggesting that sorbose may be entering by an active transport system and not by mere diffusion (Crocken and Tatum, 1967). Klingmüller and Kaudewitz (1967) found mutants which were resistant to the paramorphogenetic effects of sorbose. These were *sor*^{r}-*A* on LG VI and *sor*^{r}-*B* on LG VII, which they suggested as possible loci coding for transport enzymes, and *sor*^{r}-*C* with a hypothesized regulatory function (Klingmüller et al., 1970). Mishra and Tatum (1972) found a marked decrease in the activity of two glucan synthetases, glycogen synthetase (α-1,4-glucan) and β-1,3 glucan synthetase, involved in cell wall synthesis in sorbose-grown cultures. They showed that in the presence of sorbose there was a marked *in vivo* and *in vitro* decrease in the activity of these enzymes, while the same enzymes from the sorbose-resistant patch *(pat)* strain show no such decrease in activity. Previously, Mahadevan and Tatum (1965) had shown that sorbose alters

wild type cell wall constituents, including a sharp decrease in β-1,3-glucan, while having no affect on the cell walls of the *pat* mutant. Mishra and Tatum (1972) were also able to show that sorbose did not exert an effect on several other enzymes *in vivo* or *in vitro* including glucose-6-phosphate dehydrogenase, phosphoglucomutase, 6-phosphogluconic acid dehydrogenase, phosphoglucosisomerase, hexokinase and NADP-linked isocitrate dehydrogenase. Trinci and Collinge (1973a) reported sorbose effects in reducing hyphal diameter, size of the peripheral growth zone, and the distance between septa. In their cultures, grown on 2% sorbose plus 1% sucrose Vogel's minimal medium, they noted much smaller, more dense colonies, a reduced radial growth rate and greatly increased hyphal branching. It was also observed that growth did not occur for 10-14 days on medium containing sorbose as the sole carbon source. Hence, studies of aerialogenesis carried out on sorbose media may involve two causally different types of mutants. Some mutants may be of a sorbose-resistant type (Klingmüller and Kaudewitz, 1967; Klingmüller et al., 1970) while other phenotypic alterations are observed due to genetic effects on the aerialogenesis process.

As mentioned earlier (Table I) numerous studies have correlated a visible morphological alteration with

a specific gene mutation, a metabolic alteration, and/or a change in the chemical composition of the normally rigid cell wall. An observation relating two of these variables, such as a genetic locus, with a morphological change, might imply that alteration in the other variables was probably also occurring. From the work of Halaban (1975) and others it now appears that aerialogenesis and conidiogenesis may be separately regulated. If these two events can be separated, then aerialogenesis may not be developmentally linked to asexual reproduction, as was previously thought, and may be treated as a separate process in differentiation.

To this point in time, no reports have been published specifically attacking the problem of the genetic control of aerial extension growth. There have been no means of sufficient sensitivity available for quantitating aerialogenesis, so that different genetic components might be recognized. This study has undertaken to fill some of this gap in the understanding of differentiation in *Neurospora crassa*. First of all a technique for observing and quantitating aerial extension growth was devised. Then, computational and statistical methods were selected for transforming the observational data into a form usable for the analysis of many strains. Lastly, the transformed data were used to characterize strains and the heritable

components of their aerialogenesis phenotype.

MATERIALS AND METHODS

SECTION I. MATERIALS.

A. Notations and Abbreviations.

Strains derived prior to the author's assumption of the study carry their original notation and are described in Table 2.

All strains isolated during this study are derived from single ascospores isolated sequentially from intact asci. These strains bear an ascus number, indicating the cross involved, and a spore number, corresponding to the location of the spore in the ordered ascus, e.g. 59-4: ascus fifty-nine, the fourth of eight spores.

The following abbreviations have been used in the text:

OR -	an Oak Ridge strain or background
mt -	the mating type locus on linkage group I
A and *a* -	the two alternative alleles of the mating type *(mt)* locus on linkage group I
aer -	an allele producing aerial hyphae which will be further defined by an appropriate numbering scheme in the Discussion

TABLE 2

Stock Strains Derived Prior to this Study

Strain	Parents		Colour	M.T.	Aerial	Segregation[a] (-:+)	Auxotrophic for:
12-C	467*	469*	*ylo*	A	?	NA	*pan-2(3), trp-3*
3-C	467*	469*	*ylo*	A	?	NA	*pan-2(3), trp-3*
14-5	467*	469*	*al*	a	?	NA	*ad-3 pan-2(3), al-2*
A2	12-C	14-5	*ylo*	A	-	NA	
A9	3-C	14-5	*ylo*	a	±	NA	
OR4 (Oak Ridge)	ORA	ORa	*ylo*$^+$	a	-	NA	
OR1	ORA	ORa	*ylo*$^+$	A	-	NA	
8-1	A2	OR4	*ylo*$^+$	a	+	2:2	

TABLE 2 (continued)

Strain	Parents		Colour	M.T.	Aerial	Segregation@ (-:+)	Auxotrophic for:
8-3	A2	OR4	*ylo*$^{+}$	a	+	2:2	
8-5	A2	OR4	*ylo*	A	-	2:2	
8-7	A2	OR4	*ylo*	A	-	2:2	

* Fungal Genetics Stock Center (FGSC) strain numbers
\- aerial
\+ nonaerial
@ refers to the ascus segregation, aerial:nonaerial, in the ascus the strain was isolated from, based on the criterion of number of inocula exhibiting the maximum aerialogenesis phenotype (4) by 96 hrs (as explained in this Chapter, Section II, Part E)
NA not applicable, these strains were all random spore isolates or untested by the criteria used for this study

aer$^+$ - the wild type allele of each *aer* mutant allele, which does not contribute to aerial hyphae production under the experimental conditions

ylo - a mutant allele on LG VI associated with yellow conidial color

ylo$^+$ - the wild type allele of *ylo* which is associated with pink conidia production

TGN - Total Growth Number; the sum of all the growth readings for a strain divided by the number of times the strain was plated (explained further in Section II, Part F).

GCN - Growth Curve Number; the sum of all the growth readings for a strain, at a given time, divided by the total number of times the strain was plated (explained further in Section II, Part F)

PD - a parental ditype ascus segregation pattern (i.e. 2 ab, and 2a$^+$b$^+$ from a cross of ab x a$^+$b$^+$)

NPD - a nonparental ditype ascus segregation (i.e. 2ab$^+$ and 2a$^+$b from a cross of ab x a$^+$b$^+$)

T - a tetratype ascus segregation (i.e. lab, la$^+$b$^+$, lab$^+$ and la$^+$b from a cross of ab x a$^+$b$^+$)

LG - linkage group, in *Neurospora crassa* accompanied by a roman numeral from I to VII, designating one of the seven linkage groups

pan-2(3) or *pan-2(5)* - two different mutant alleles (separable by recombination) at the *pan-2* locus on LG VI associated with pantothenic acid auxotrophy (although they may be supplemented with anthranilic acid)

pan-2(3)$^+$ or *pan-2(5)*$^+$ - the wild type alleles at each of these sites within the *pan-2* locus which are associated with pantothenic acid prototrophy

tryp-2 - a mutant allele on LG VI associated with tryptophan auxotrophy

tryp-2$^+$ - the wild type allele of *tryp-2* associated with tryptophan prototrophy

ad-1 - a mutant allele on LG VI associated with adenine auxotrophy

ad-1$^+$ - the wild type allele of *ad-1* which is associated with adenine prototrophy

al-2 - a mutant allele on LG I associated with unpigmented conidia

al-2$^+$ - the wild type allele of *al-2* which is associated with pink conidia production

50X - salt solution which was diluted 50 times for use in media

B. Strains Used in This Study.

Strains used during the course of this study are shown in Tables 2 and 3. The parents were selected arbitrarily while the progeny strains were selected for their aerialogenesis phenotype.

Stock strains were maintained on 1 ml slants of solid glucose medium at -17°C.

C. Media.

All media contained an inorganic salt solution, henceforth designated 50X, described by Vogel (1956). This solution was made up at fifty times strength and stored at room temperature in sealed flasks. The 50X salt solution consisted of:

$Na_3C_6H_5O_7 \cdot 2\ H_2O$	125 g
KH_2PO_4	250 g
NH_4NO_3	100 g

TABLE 3

Strains Derived During this Study

Strain	Parents		Colour	M.T.	Aerial	Segregation*(-:+)
59-1	A2	OR4	*y lc*	A	+	3:1
59-4	A2	OR4	*y lc*	A	-	3:1
59-5	A2	OR4	*y lc*$^+$	a	+	3:1
59-8	A2	OR4	*y lc*$^+$	a	-	3:1
275-3	8-1	59-4	*y lc*	A	-	2:2
240-1	8-1	59-4	*y lc*$^+$	A	+	0:4
240-3	8-1	59-4	*y lc*$^+$	A	+	0:4
240-5	8-1	59-4	*y lc*	a	+	0:4
240-7	8-1	59-4	*y lc*	a	+	0:4
131-5	8-1	8-5	*y lc*	a	-	4:0
131-7	8-1	59-4	*y lc*	A	-	4:0

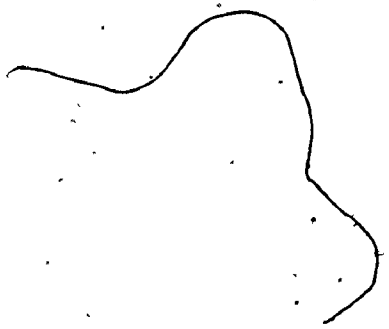
TABLE 3 (continued)

Strain	Parents		Colour	M.T.	Aerial	Segregation*(-:+)
173-7	8-1	8-5	*ylo*$^+$	A	+	3:1

* refers to the ascus segregation, aerial:nonaerial, in the ascus the strain was isolated from, based on the criterion of number of inocula exhibiting the maximum aerialogenesis phenotype (4) by 96 hrs (as explained in this Chapter, Section II, Part E)

\+ nonaerial

\- aerial



$MgSO_4 \cdot 7\ H_2O$	10 g
$CaCl_2 \cdot 2\ H_2O$	5 g
trace element solution	5 ml
Biotin solution	250 µg
Chloroform	3 ml
Distilled water	750 ml

The trace element solution for the 50X salt solution (Vogel, 1956) consisted of:

citric acid $\cdot 1\ H_2O$	5.0 g
$ZnSO_4 \cdot 7\ H_2O$	5.0 g
$Fe(NH_4)_2\ (SO_4)_2 \cdot 6\ H_2O$	1.0 g
$CuSO_4 \cdot 5\ H_2O$	0.25 g
$MnSO_4 \cdot 1\ H_2O$	0.05 g
H_3BO_3	0.05 g
$Na_2MoO_4 \cdot 2\ H_2O$	0.05 g
Distilled water	95 ml

Crosses were made on modified Westergaard and Mitchell (1947) medium consisting of 2% sucrose as the carbon source, 1.5% Bacto-Difco agar, distilled water, plus 50X to give the appropriate final concentration.

For vegetative growth, Vogel's (1956) medium was used with 2% glucose as the carbon source solidified with 1.5% Bacto-Difco agar.

The medium employed for testing strains on plates

consisted of 50X salt solution, 0.05% fructose, 0.05% glucose, and 1.0% sorbose (Brockman and de Serres, 1963) solidified with 2% Bacto-Difco agar.

All media were autoclaved at 15 psi above atmospheric pressure (251°C) for fifteen minutes. Care was taken with the plating medium to avoid heat decomposition of the sorbose by not surpassing the fifteen minute time limit and by using a small autoclave which was able to come down faster.

SECTION II. FUNGAL METHODS

A. Crosses.

Crosses were carried out in 150x15 mm test tubes on 5 ml slants of the sucrose-crossing medium. An inoculum of the protoperithecial parent was allowed to grow for five to seven days at 25°C before addition of the conidial parent as a distilled water suspension of a vegetative culture of the same age. All crosses were incubated at 25°C.

B. Isolation of Asci.

Crosses varied greatly in length of time taken to discharge the majority of their spores. Isolation of asci was started as soon as possible after spores were first noticed on the wall of the tube.

Perithecia were isolated to drops of sterile

distilled water on a flamed microscope slide and were broken open using sterile tungsten wire needles. Rosettes of asci possessing mature black ascospores were transferred with a needle from the slide to a block of 4% agar on another slide. The rosettes were broken apart to contain fewer, but usable, asci per clump either on the original slide or on the agar block and then allowed to dry. At the appropriate stage of dryness, single asci were dragged to an empty area on the agar block and allowed to dry further. Individual ascospores were then isolated in order at 35X magnification, using tungsten wire needles sharpened by oxidation in molten sodium nitrite. Each spore was transferred from the tip of the needle to a small tube containing a 1 ml slant of glucose-vegetative medium.

A record was kept of spore colour for individual isolated spores, when not black, and of possible discrepancies in the order of isolation.

Asci were stored at 25°C for ten to fourteen days to allow time for maturation. Sterile distilled water was then added to compensate for water loss during storage, and the asci were heat shocked at 60°C for approximately 43 minutes in a water bath. Further heat shocks were carried out as necessary, although few spores were seen to germinate after the second or third heat shock.

C. Determination of Mating Types.

Mating type tests were carried out by inoculating two small tubes of modified Westergaard and Mitchell medium (sucrose-reproductive medium) with the strain to be tested. Within 24 hrs a conidial suspension of an Oak Ridge strain of known mating type was added to each tube, *mt* A in one tube, *mt* a in the other. Mating type was scored after at least two weeks when perithecia and discharged ascospores could be seen clearly in one of the tubes and not the other.

D. Determination of Aerial Hyphae Types.

Determination of aerial hyphae types was carried out in 9 cm. disposable petri dishes using approximately 22 ml of the sorbose-plating medium described above. Each of the strains isolated from two asci and their parent strains were inoculated with a wire twist on each plate. All plates were incubated, with the medium on top, in growth chambers set for a minimum of 60% relative humidity (the humidity control was only for ambient and above, hence when ambient was greater than 60%, the experimental humidity was equal to the ambient humidity). Twenty replicate plates were incubated at 32°C and 0, 10, or 20 plates were incubated at 25°C, depending on the experiment.

All testing of strains was carried out in the

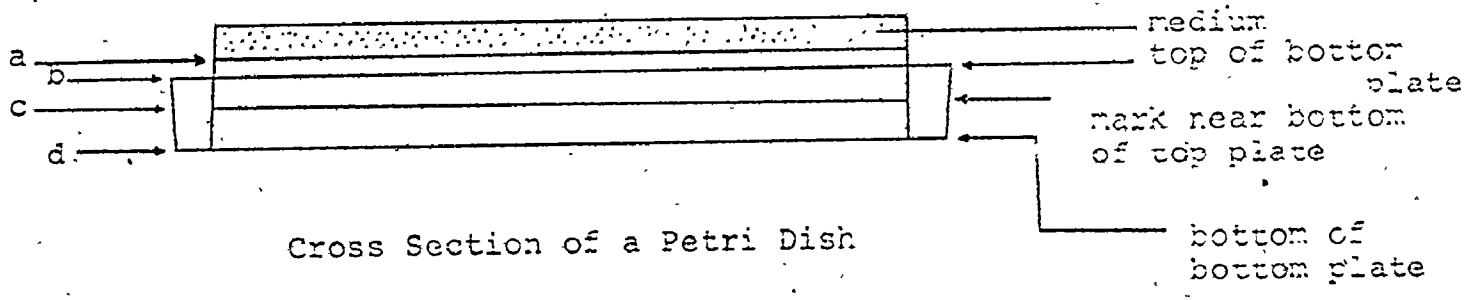
dark except for a lighted period of approximately 5 min duration for each plate at each of the seven observation times. The amount of aerial hyphae growth was estimated at 48, 54, 60, 72, 78, 84, and 96 hours after inoculation. The estimation procedure was facilitated by divisions present in the construction of each petri dish (Figure 1). Inocula without aerial growth were scored as 0. Those with hyphae between a and b were scored as 1. Those between b and c as 2 and those between c and d, without touching d, as 3. The colonies possessing hyphae which had grown all the way from the medium to the bottom plate were classified as 4 (Table 4).

E. Preliminary Analysis of Aerialogenesis Observations.

One of the major problems in this study has been the characterization of the genetic component of aerial growth in the strains being tested. In the early stages of this study less complex methods of data analysis were employed to distinguish between loci of major effect in the production of aerial hyphae. This technique was sufficient for loci of major effect but proved too insensitive for genes of lesser effect. More complex procedures of data analysis were then undertaken to detect loci associated with the reduced aerialogenesis visible in later experiments.

FIGURE 1

Classification of Aerial Types



Cross Section of a Petri Dish

TABLE 4

Classification of Aerial Hyphal Types

Aerial Hyphal Type	Height as Indicated on Petri Dish
1	$> a \leq b$
2	$> b \leq c$
3	$> c \cdot d$
4	$= d.$

The system of classification described in Part D of this Section (1 being the least aerial growth and 4 being the most aerial growth) was used throughout the course of this study. The preliminary method of analysis involved comparing the number of inocula of a strain which gave maximum aerial growth (4) by 96 hrs with the same criteria for other strains. An example of this method would be to assume each of two strains were plated twenty times. If one of the strains had no colonies exhibiting maximum aerial growth by 96 hrs (0/20 showing aerial phenotype 4) and the other strain had all twenty inocula exhibiting the maximum aerial growth phenotype (4) by 96 hrs, then the first strain would be classified as nonaerial by this criteria, and the second strain as aerial. This method proved sufficient for identifying the aerial producing loci of major effect found in this study. Strains could be classified as being aerial or nonaerial based on this criteria and segregation patterns of aerial:nonaerial could be assigned for each ascus. This method was only of significant value where aerial phenotypes were near the two extremes, maximum aerialogenesis or none at all. In later crosses, where loci with less effect on aerialogenesis were being studied, this method proved too insensitive for classifying

a majority of the progeny and more complex methods were utilized to analyze the later data (these methods are explained in Part F of this section).

F. Total Growth Numbers and Growth Curve Numbers

From a single inoculum, grown for aerial hyphae observation, seven readings are taken (this Section, Part D). Each reading provides a figure between 0 and 4, depending on the extent of aerial hyphae growth. The seven readings are made at seven different times between 48 and 96 hrs (48, 54, 60, 72, 78, 84, and 96 hrs). The seven numbers, so obtained from a single inoculum, are summed to yield a value known as a Total Growth Number (TGN), in this case, an inoculum TGN. These TGNs are calculated for all replicate inocula of a given strain (usually 20 replicates per strain). I call the arithmetic mean of the inoculum TGNs for a strain, the strain Total Growth Number. Significant differences between strain TGN values for spore pairs obtained from asci are usually absent, in which case the TGN values are combined for all inocula of a spore pair to give one TGN value for the spore pair data which is known as the spore pair TGN value. Thus TGNs may be calculated for inocula, strains or spore pairs. TGN values represent both the amount of aerial growth, and the time of its initiation.

Another value, derived from a different way of looking at the observational data, is designated the Growth Curve Number (GCN). GCNs are averages (arithmetic means) of the growth values (0-4), for each of the seven observation times (between 48 and 96 hrs). GCNs are used in plotting growth curves for each strain. The GCN values for a strain, when summed, are equal to the TGN value for that strain. GCN values may also be plotted for spore pair data where significant differences between the spore pairs are absent.

Both TGN and GCN values are discussed further in Section III of this Chapter and in the Appendix.

G. Assays of Conidiation.

Strains selected with characteristic growth types in large numbers of tests were further tested for the type of conidiation and relative numbers of conidia produced at various time periods at 25°C or 32°C on glucose or sorbose media. Microconidiation, involving the production of uninucleate conidia, occurs on relatively shorter clusters of conidiophores along the surface of the medium. Macroconidiation, which produces conidia containing a variable number of nuclei, is usually associated with conidiophores on relatively longer aerial hyphae (Grigg, 1960a).

Nuclear counts per conidium were carried out using a Giemsa staining method for conidia isolated from several strains selected as before. These strains were grown in small tubes of glucose or sorbose media at 25°C or 32°C. At each assay time, mycelia and conidia were taken from the small tubes and suspended in phosphate buffer at pH 6.4 or spread in a drop of the buffer on a microscope slide. The buffer used contained KH_2PO_4 and K_2HPO_4 in approximately 0.1 M solutions adjusted to pH 6.4. The slide was allowed to air dry and then fixed by immersion in a 1:3 (vol:vol) glacial acetic acid:alcohol for ten minutes. The slide was then washed several times in distilled water and heated for four to five minutes in 5N HCl at 60°C. The slide was washed again in distilled water and covered with Gurr's R66 Giemsa Stain (G. T. Gurr Ltd., London, S.W. 6, England, supplied by ESBE Laboratory Supplies, 459 Bloor St., Toronto 4, Ontario) for two to three minutes. The stain was a 1:9 (vol:vol) solution of Gurr's Giemsa: Gurr's pH 7.0 Buffer. The slide was washed and allowed to air dry before making counts of nuclei per conidium using an oil immersion lense on a compound microscope. In each sample but one, over 100 conidia were scored for nuclei per conidium and the results

recorded. This analysis was used as an indication of the relative amounts of micro and macroconidiation under the various conditions.

For quantitative analysis of conidia production, strains were inoculated on plates in the same manner as described earlier for the analysis of aerialogenesis (this Section, Part D). At each time interval studied, small tubes containing 3 ml of distilled water were placed under each colony on the plate. The plate, plus all the tubes, each containing a colony and the agar adjoining it, and the tube holder, were manually agitated to shake off conidia. These solutions were vortexed and samples taken and placed on standard hemocytometer slides for counting conidia. All counting was done on the same type of slide and the total number of conidia per hemocytometer field was recorded for a number of replicates for each tube as well as other inocula of the strain, collected in different tubes. These values were then used for comparisons of relative amounts of conidiation for the strains tested.

SECTION III. COMPUTER ANALYSIS.

A. Computer Program Abbreviations.

The following list of computer abbreviations was

used in construction of the program and will be referred to in the discussion of the computer analysis:

IDATA - a three dimensional array containing the data of two asci and their two parents (Figure 2)

GCN - a two dimensional array containing GCN's for each strain (Section III, Part C).

NUM - total number of inoculations for each strain (counts number of replicate inocula for a strain)

TGN1 - a two dimensional array containing inoculum TGN's for each strain

TGN2 - summed TGN1's for each strain (the sum of the observations for a strain)

TGN3 - sum of squared TGN1's for each strain (sum of the squares of the inocula TGN's for a strain)

AMEAN - mean TGN1 for each strain (referred to as TGN in the text) (Section III, Part D)

SD - standard deviation of each AMEAN

SE - standard error of each AMEAN

STUDT1 - Student's t value for similarity of spore pairs based on TGN1 values

STUDTP - Student's t value for similarity of first member of spore pair with first parent

STUDTQ - Student's t value for similarity of first member of spore pair with second parent

STUDT2 - Student's t value for similarity of second member of spore pair with first parent

STUDT3 - Student's t value for similarity of second member of spore pair with second parent

NUM2 - total number of inoculations for both members of a spore pair (counts number of replicate inocula for a spore pair)

AMEAN2 - mean TGN1 value for the lumped spore pair data (Section III, Part D)

TGN4 - sum of TGN1's for spore pairs

TGN5 - sum of squared TGN1's for spore pairs

SD2 - standard deviation of spore pair AMEAN2

GCN1 - two dimensional array containing the sum of GCN's for each strain at each time

GCN2 - two dimensional array containing the sum of squared GCN's for each strain at each time

SD3 - standard deviation for each strain GCN at each time

SE1 - standard error for each strain GCN at each time

STUDT4 - Student's t value for spore pair TGN compared to first parent

STUDT5 - Student's t value for spore pair TGN compared to second parent

GCNS - a two dimensional array containing GCN values for spore pairs

Indices (Figure 2)

(I) - I=1 is the first parent, I=2,...,9 is the first ascus, I=10 is the second parent, I=11,...,18 is the second ascus

(M) - M=1,...,4 the four spore pairs of the first ascus, M=5,...,8 four spore pairs of the second ascus

(J) - J varies from 1 to 7; J=1 is the 48 hr time reading, J=2 is the 54 hr time reading,...J=7 is the 96 hr time reading

(K) - K indexes the twenty possible replicates

B. Programming and Data Handling.

Due to the large volume of data generated in

the experiments, over 325,000 observations at 32°C, even the simplest calculations required a great deal of time under standard desk calculator procedures. To allow for a significantly greater sophistication of analysis the McMaster University computer facility, a CDC 6400, was utilized. I created a standard analysis program for my data in the Fortran IV language with the assistance of Dr. R. Morton, of the Department of Biology, and Dr. P. Macdonald and Professor K. Redish, of the Department of Applied Math. Several publications were also helpful (McCracken, 1972; Maurer and Williams, 1972; Veldman, 1967; and Bayley, 1975) in developing a reasonably efficient and usable program for my data. All of the calculations were carried out on 32°C observations. Normally, the 25° colonies produced insufficient growth for an adequate aerialogenesis characterization during the time of these experiments. Hence, there were no consistent differences found at 25°C and the 25°C platings were discontinued in later experiments.

Each petri dish contained 18 inocula : the two parent strains, plus two progeny asci (8 spores each). The growth number of each inoculum was observed at 7 different times (48h, 54h, etc.). Hence each petri dish provided 7 x 18 = 126 growth numbers, each between

0 and 4. In addition, each plating was replicated 20 times. Thus the keypunched data were read into the FORTRAN array IDATA (I,J,K), where I indexes the 18 strains and J the 7 observation times on a given petri dish, and K indexes the 20 replications of that plating. The 18 inocula were ordered so that I=1 corresponds to the first parent, I=2,...,9 corresponds to the 8 spores of the first ascus, I=10 corresponds to the second parent, and I=11,...,18 corresponds to the 8 spores of the second ascus. The array thus contains 2520 observed growth numbers (18 inocula x 7 observation times x 20 replicates) for a given cross. Where there was an inoculum missing or a spore lost seven number nines were punched on the data card so that an accurate count of NUM and NUM2 would be kept. The arrangement of the data in the IDATA array, including the arrangement of (M) values is shown in Figure 2.

C. Growth Curve Numbers.

The Growth Curve Number (GCN) for a strain, at a given time, is defined as the sum of all the numerical observations for inocula of that strain at that time (I and J constant, K=1,...,20, divided by the total number of times the strain was plated (NUM) (explained further in Section II, Part F). GCN values varied from

Figure 2. Diagram of the IDATA(I,J,K) three dimensional array including index (M).

	J	1	2	3	4	5	6	7
	TIME	48	54	60	72	78	84	96
M	I	INOCULA						
1	1	p1						
	2	a1-1						
	3	2						
2	4	3						
	5	4						
3	6	5						
	7	6						
4	8	7						
	9	8						
	10	p2						
5	11	a2-1						
	12	2						
6	13	3						
	14	4						
7	15	5						
	16	6						
8	17	7						
	18	8						

This pattern is repeated for K=1,...,20

p= parents, first or second, as designated
a= ascus, first or second, as designated, followed by the number of each spore in the ascus

a minimum of 0 (no growth of the strain at the time) to a maximum of 4 (maximum aerial growth for all inocula of the strain at the time in question). Hence an array of GCN values can be calculated from the data and stored in the array GCN(I,J). Standard deviations (SD3) and standard errors (SE1) (Sokal and Rohlf, Chap 4, 1969; Bishop, Chap. 3, 1971; Schefler, Chap 4, 1969) were also calculated for the GCN's, as an estimate of the homogeneity of the samples. The GCN's, with their SD3 and SE1 values, were printed in the computer output, and the GCN's were also used to plot growth curves for each strain tested. This allowed for both numerical and graphical comparisons of growth of the various strains and their parents. When no significant differences were found between spore pairs based on TGN values the spore pair data were combined for GCN values, creating a new array of values (GCNS) with eight spore pairs per plating (M) and the same seven time intervals. These data, together with the parental data, were used to plot growth curves for the spores and parents. This latter type of plot of GCN values proved most useful, in tandem with statistics to be described in the following section, in characterizing the growth of the strains being tested.

D. Total Growth Numbers.

The Total Growth Number (TGN) for a strain was calculated by summing all the growth number observations (I constant, J=1,...,7, K=1,...,20) for the inocula of a strain and dividing by the total number of times plated (NUM) (Figure 2) (explained further in Section II, Part F). This number was a reflection of both the time aerial growth began and the amount of aerial growth observed. The minimum TGN is 0 and the maximum is 28 (maximum growth number of four at each of the seven times). The strains of each spore pair were compared to each other (STUDT1) and to each parent (STUDTP, STUDTQ, STUDT2, STUDT3) by Student's t-test (Sokal and Rohlf, Chap 7, 1969; Rohlf and Sokal, Table Q, 1969). The standard deviation (SD) and standard error (SE) were calculated for each strain and these values were printed in the output along with TGN values of each inoculum of each strain. Student's t values within spore pairs were consistently low (no significant differences), therefore the TGN data for spore pairs were analyzed (AMEAN2(M=1,...,8) and standard deviations were calculated for the combined data (SD2). The combined spore pair data were also compared to each parent by Student's t-test (STUDT4, STUDT5). NUM2 was equal to the total number of observable colonies from the two members of the spore

pair (for M=1, NUM2(1)= NUM(2) + NUM(3)).

The variation between different inocula of the same strain was consistently low over the course of the experiment, as determined by comparing standard deviations and variances for strains. The variation between the genetically identical members of spore pairs was also found to be very low when the Student's t-test was used to compare the means. On several occasions, the lab technician scored sets of plates at certain time intervals for aerialogenesis phenotype. When the results obtained by the technician were compared to those obtained by myself, there was still consistency between spore pairs. This consistency between the results obtained by two individuals using this technique indicates that the data were relatively free from bias due to operator error.

A sample program with a more complete explanation of the output for two asci and their parents will be found in Appendix A.

RESULTS

In the Materials and Methods Chapter, Section II, Part D, I have described an original technique used throughout this study for quantifying aerial extension growth (with 0 being no aerial growth and 4 being maximum aerial growth). The Materials and Methods Chapter also describes two original methods of analyzing the data gathered so that it would better represent the aerial growth of a strain. The less complex of these methods was described in Section II, Part E and involved the comparison between strains of the number of inocula exhibiting the maximum aerial growth phenotype (4) by 96 hrs. The other technique is described in Materials and Methods, Section II, Part F. This technique was applied to the data via a computer analysis (Materials and Methods, Section III and Appendix A), which generated several useful numerical comparisons, including GCN, TGN and the accompanying Student's t-test calculations, as well as allowing graphical comparisons of the GCN plots between strains. The less complex analysis was employed in the earlier crosses and will be used at the beginning of this chapter. Later in this chapter the computer analysis of the data will be used to describe the observed aerialogenesis

phenotypes.

Figure 3 summarizes the crosses made during this study, including the phenotypes of the parental strains and the segregation patterns in the progeny. The phenotypes were determined using the criteria of number aerial by 96 hrs, as explained in Materials and Methods, Section II, Part E. The two original parents, A2 and OR4 both had aerial phenotypes. From the first cross, ascus segregation patterns of 4:0, 3:1, and 2:2 for aerial:nonaerial were observed (Table 5), indicating the segregation of more than one locus associated with the aerial phenotype. Ascus 8, an ascus from this cross with a 2:2 segregation pattern, was one of the asci chosen for further analysis. This further analysis entailed crossing aerial and nonaerial strains from ascus 8 and observing ascus segregation patterns in the progeny (crosses 3-6, Table 5). Figure 4 shows a plot of the parents and progeny of reciprocal crosses using strains 8-1 and 8-5 employing the aerial by 96 hrs criteria. The data from 26 asci (208 progeny strains, essentially treated as random spores) show a distinctly bimodal distribution between those strains similar to the 8-1 parent (the open squares), which show very few or no completely aerial colonies by 96 hrs, and strains similar to the 8-5 parent (the open triangles), which show from about one third of all of their inoculations with complete aerial growth by 96 hrs. In Figure 4, for

FIGURE 3

A Summary of the Crosses Made During this Study and the Observed Ascus Segregation Patterns for aerial:nonaerial Growth Using the Criteria of Numbers Aerial (4) by 96 hrs.

A2 (aerial) - OR4 (aerial)
4:0, 3:1, 2:2
(59) (8)

59-1 (aerial) - 8-1 (nonaerial)
4:0,3:1,2:2

59-4 (aerial) x 8-1 (nonaerial)
2:2,1:3,0:4
(240) Types II and III*

8-1 (nonaerial) x 59-4 (aerial)
2:2,1:3,0:4
(275) Type I*

59-5 (nonaerial) - 275-3$^{\pm}$ (nonaerial)
0:4

59-8 (aerial) - 275-3$^{\pm}$ (nonaerial)
2:2

8-1 (nonaerial) - 8-5 (aerial)
4:0,3:1,2:2
(131)

8-1 (nonaerial) - 8-7 (aerial)
4:0,3:1,2:2

8-3 (nonaerial) - 8-5 (aerial)
4:0,3:1,2:2

8-3 (nonaerial) - 8-7 (aerial)
4:0,3:1,2:2

FIGURE 3 (continued)

275-3± (nonaerial) x 8-1 (nonaerial)
0:4

240-1 (nonaerial) x 240-5 (nonaerial)
2:2,1:3,0:4

240-1 (nonaerial) x 240-7 (nonaerial)
2:2,1:3,0:4

240-3 (nonaerial) x 240-5 (nonaerial
2:2,1:3,0:4

240-3 (nonaerial) x 240-7 (nonaerial)
2:2,1:3,0:4

131-5 (aerial) - 8-1 (nonaerial)
2:2

131-5 (aerial) - 173-7° (nonaerial)
2:2

* separate Types of asci to be explained in the Discussion
± 275-3 is a nonaerial tester strain derived from a Type I ascus from cross 59-4 x 8-1
° 173-7 is a nonaerial tester strain derived from a 3:1 ascus from a cross using strains 8-1 and 8-5 as parents.
- between strains indicates reciprocal crosses
x between strains indicates the protoperithecial parent first

Figure 4. Parents, 8-1 and 8-5, and their progeny, showing numbers of inocula with maximum aerial growth (4) by 96 hrs from sets of 20 inocula.

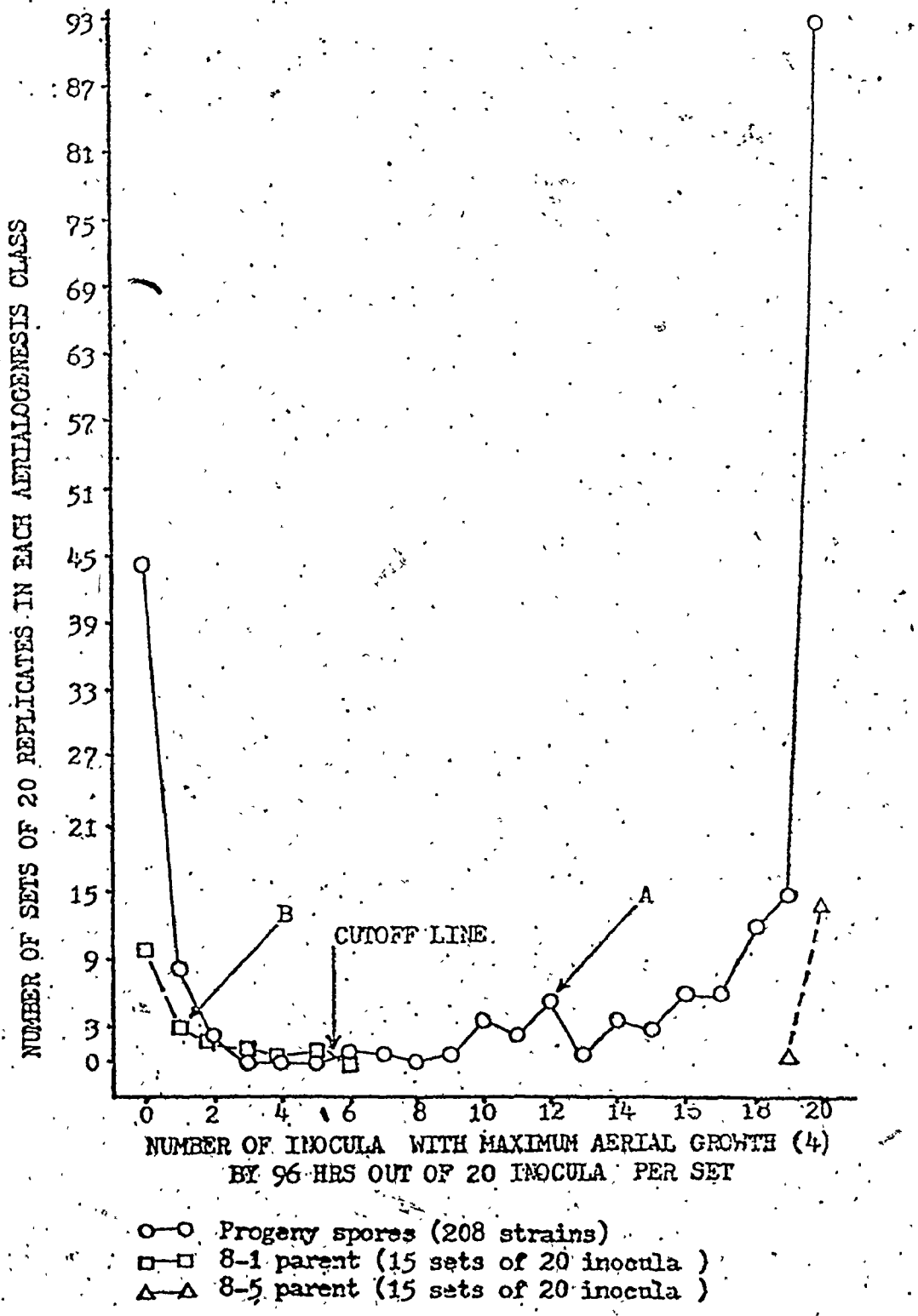


TABLE 5

Ascus Segregation Patterns Using the Criteria of Maximum Aerial Growth (4) by 96 hrs.

#	Cross	Ascus Segregation aerial:nonaerial					Total Asci	% Germination
		4:0	3:1	2:2	1:3	0:4		
1	A2 - OR4	1	10 (59)	5 (8)			16	>90
2	OR4 - 8-5	8					8	>95
3	8-1 - 8-5	3 (131)	19 (173)	4			26	>90
4	8-1 - 8-7	2	7	7			16	>90
5	8-3 - 8-7	2	16	2			20	>90
6	8-3 - 8-5	2	7	3			12	>95
7	59-4 - 8-1			20 (275) *	4	2 (240)	26	>95
8	59-1 - 8-1	3	13	6			22	>95
9	59-8 - 275-3			14			14	>95

TABLE 5 (continued)

#	Cross	Ascus Segregation aerial:nonaerial					Total Asci	% Germination
		4:0	3:1	2:2	1:3	0:4		
10	59-5 - 275-3					12	12	>95
11	8-1 x 275-3					8	8	>90
12	131-7 x 8-1			10			10	>99
13	131-5 - 173-7			12			12	>90
							202	

(#) indicates source of strains with this ascus number used for further analysis
x # indicates protoperithecial parent first
- # indicates crosses were done reciprocally and when no differences were found all progeny asci from the two crosses were lumped (in this analysis no differences were found in cross 7, but under more sensitive tests differences became apparent)
aerial:nonaerial determination based on plots for each cross similar to Figure 3
*275 from Type I asci which do not exhibit the NG phenotype (explained further in Discussion)

instance, three of the fifteen replicates, of twenty inocula each, for strain 8-1, contained one out of the twenty colonies exhibiting maximum aerial growth (4) by 96 hrs (Point B). Point A in Figure 4 indicates, that of the 208 progeny strains, which were each inoculated twenty times, five strains had twelve of their twenty colonies exhibiting maximum aerial growth by 96 hrs. Similar plots were carried out for all of the crosses with loci of major effect segregating in the parents (crosses 1-9,12,13) and a table of these results, together with several control crosses follows (Table 5). The percent germination is also given in Table 5 as an indication that no large classes were being lost preferentially. The only asci which were tested had either seven or eight viable spores so that any inconsistencies between members of spore pairs might be seen. The two members of a spore pair always fell on the same side of the cutoff line (Figure 4) separating the parental types.

The segregation of loci producing a major effect on aerialogenesis was also compared to the segregation of their centromeres and to two known marker loci (*ylo/ylo*$^+$ and *A/a*). Table 6 shows the results obtained when comparing the two crosses with one major aerial locus segregating. An absence of linkage between this aerial locus and the *ylo* locus is indicated by the similarity in frequencies

TABLE 6. Results of Crosses with one Major Aerial Locus Segregating.

CROSS	PARENTS		Aerial Segregation				
			PD	NPD	T	1st div.	2nd div.
9	59-8	275-3	1	0	13	2	12
12	131-7	8-1	0	1	9	1	9
			1	1	22	3	21

Parental ditype (PD), Nonparental ditype (NPD) and Tetratype (T) are all in respect to the parental growth types using the criteria of maximum growth by 96 hrs and *ylo/ylo*$^+$ conidial color.

of parental ditype (PD) and nonparental ditype (NPD) asci. The large number of tetratypes (T) also indicates that this locus is far from its centromere while the *ylo* locus is known to be close to the centromere on the left arm of linkage group VI (Fincham and Day, 1971). By similar means this locus can also be shown to behave as if unlinked to the mt locus.

From the 4:0 and 3:1 segregations shown in Table 5 (crosses 3-6,8) it is evident that there is more than one genetic means of producing the maximum amount of aerial growth in the experiment. A single locus effect has been shown previously (Table 6). The results of reciprocal crosses of strains 59-4 and 8-1 (Table 7) show the segregation of another aerialogenesis capability in relation to the *ylo* and mating type loci. The single locus was not segregating its aerial producing allele in this cross (Table 7) and hence, its potentially confounding effect was not observed. From Table 7, the parental loci for aerial production can be seen to be linked to the *ylo* locus based on the larger numbers of PD asci while the loci appeared unlinked to the mt locus by the small number of PD asci. Segregations of 4:0, 3:1 and 2:2 for aerial:nonaerial in 3, 4, 5, 6, and 8, where aerial and nonaerial parents were used, indicated more than one locus was involved in the production of the maximum aerialogenesis. Cross 7 (Table 5) indicated that one of these aerial producing genotypes

TABLE 7. Ascus Types from Reciprocal Crosses with Strains 8-1 and 59-4 (cross 7).

Characters Segregating	PD	NPD	TT	TOTAL
parental aerial vs *ylo/ylo*$^+$	12	3	11	26
parental aerial vs MT	1	5	14	20*

* only 20 of the 26 asci had mt tested.

could undergo segregation or recombination. This segregation or recombination led to less than one half of the progeny in an ascus possessing the aerial phenotype similar to that of the aerial parent. One of the asci from cross 7 (Table 5), which gave a 0:4 segregation for aerial:nonaerial, was analyzed further and the results will be presented later together with the more sensitive methods of analysis that were required.

Some recent papers (Matsuyama et al., 1974, Siegel et al., 1968, and others) have indicated an experimental design which temporally separates aerialogenesis from conidiogenesis and conidiophore production. Several strains were chosen from my study to measure both type and quantity of conidiation (Materials and Methods, Section II, Part G). The microconidia, containing only one nucleus, are thought to arise from short conidiophores, while macroconidia, with more than one nucleus, arise from longer conidiophores (Grigg, 1960, a and b). My observations of genetic effects on aerialogenesis might be correlated with changes in amounts of micro- and macroconidiation. To test this possibility, two strains were chosen which had each been well characterized for aerialogenesis. Strain 8-1 was relatively nonaerial by all criteria discussed so far and possessed *ylo*$^+$ conidia and mt *a*. Strain 8-5 was able to produce profuse aerial growth, contained a full complement

of aerial alleles, and had *ylo* conidia and mt A. These two strains were tested at various times from 24 to 192 hrs for the number of nuclei per conidium on both glucose and sorbose media at 25°C and 32°C. The resulting values of average number of nuclei per conidium, based on 100 or more conidia for each estimate but one, are very similar in all cases even though the two strains have very different morphology on sorbose media at 32°C (Table 8). From this experiment it would appear that the aerialogenesis differences observed are not correlated with type of conidiogenesis even though the aerialogenesis loci present do have a significant effect on aerialogenesis.

The possibility of correlating aerialogenesis to overall levels of conidiogenesis was investigated by counting numbers of conidia produced by selected strains at various times. Table 9 indicates the average numbers of conidia per hemocytometer field for ascus 59 and the parents, A2 and OR4, which were both aerial in phenotype. The first four and last two spores in ascus 59 were all of equal aerial production while 59-5 and 6 were both nonaerial by the criteria mentioned so far. For this ascus, there is no apparent correlation between amount of aerialogenesis and amount of conidiogenesis. This does not rule out the possibility that the loci being studied may also affect conidiogenesis.

TABLE 8

Average Numbers of Nuclei per Conidium for Strains 8-1 and 8-5 at Various Times Between 24 and 192 hrs on glucose or sorbose medium at 25°C or 32°C.

	8-1				8-5			
	GLUCOSE		SORBOSE		GLUCOSE		SORBOSE	
	25°C	32°C	25°C	32°C	25°C	32°C	25°C	32°C
HOURS								
24	3.36 (107)	3.84 (239)			3.30 (158)	3.17 (257)		
48	3.15 (259)	3.03 (241)	3.14 (22)	3.35 (405)	3.68 (233)	3.49 (219)	3.21 (180)	3.14 (172)
72	3.29 (240)	3.21 (252)	3.32 (107)	3.19 (301)	3.56 (260)	3.40 (314)	3.19 (100)	3.58 (251)
96	3.44 (221) 3.34 (154)	2.98 (213)			3.58 (208) 3.49 (148)	3.44 (210)		

TABLE 8 (continued)

	8-1				8-5			
	GLUCOSE		SORBOSE		GLUCOSE		SORBOSE	
	25°C	32°C	25°C	32°C	25°C	32°C	25°C	32°C
HOURS								
120	3.34 (237)				3.84 (244)			
144	3.15 (253) 3.74 (215)	3.11 (248)		3.20 (262)	3.31 (271) 4.02 (215)	3.20 (235)	2.83 (255)	3.14 (236)
168	3.12 (243) 3.88 (221)	2.98 (250)		2.95 (241)	3.29 (240) 3.61 (315)	3.07 (375)	2.82 (236)	2.99 (244)
192	3.36 (232)		3.12 (151)	3.26 (241)	3.50 (382)		3.42 (232)	3.30 (134)

Standard Deviation values for Nuclei/Conidium counts of strains under various growth conditions varied between 1.07 and 1.39 while the numbers of nuclei per conidium varied from 1 to 6. The maximum percentage of microconidia was 14.5% while the minimum was 1.3% for any strain under one set of conditions. The percentage of

TABLE 8 (continued)

uninucleate conidia did not vary in a correlated way with any of the known variables (i.e. time, strain, temperature). Numbers in brackets indicate the numbers of conidia examined to generate the mean value shown.

TABLE 9. Seventy-two and Ninety-six Hour Counts of Conidia per Hemocytometer Field for Ascus 59 and the Parents, A2 and OR4, Grown on Sorbose Minimal Medium.

STRAIN	72 hrs	(# of counts)	96 hrs	(# of counts)
A2	101.8	(27)	171.9	(10)
OR4	23.4	(27)	17.2	(10)
59-1	145.0	(52)	181.5	(21)
59-2	118.2	(51)	249.8	(20)
59-3	155.7	(52)	260.7	(20)
59-4	173.9	(50)	237.7	(20)
59-5	11.3	(52)	5.1	(20)
59-6	3.9	(52)	3.4	(20)
59-7	2.3	(52)	3.7	(20)
59-8	4.3	(52)	1.4	(20)

The number in brackets indicates the number of separate counts contributing to the average value given.

The strains of ascus 240 were also examined for conidiogenesis by performing double inoculations of all possible combinations of strains of the same mating type. Table 10 presents conidial counts from double inocula on sorbose medium scored at 120 hrs, plus single inoculations of the parent strains grown under the same environmental conditions. By performing double inoculations I hoped to see if there were any differences between coincident growth of spore pairs versus non-spore pairs. Any differences might have reflected genetic differences at conidiogenesis loci between certain of the spore pairs. Similarities among the first six colonies and among the second six colonies, whether or not a heterokaryon was formed, would indicate a first division segregation pattern for the conidiogenesis loci in this cross. A quantitative genetic effect on conidiogenesis appears to be segregating at first division. This effect is of comparable magnitude to the effect seen in the two parent strains and in ascus 59, shown in Table 9.

When the results were being collected for cross 7 (Table 5), it became apparent that as proportionately fewer of the progeny met the criterion of maximum growth by 96 hrs, a different, more sensitive, method of analysis would be desirable for analyzing the now more frequent nonaerial spore pairs in the data. As fewer aerialogenesis

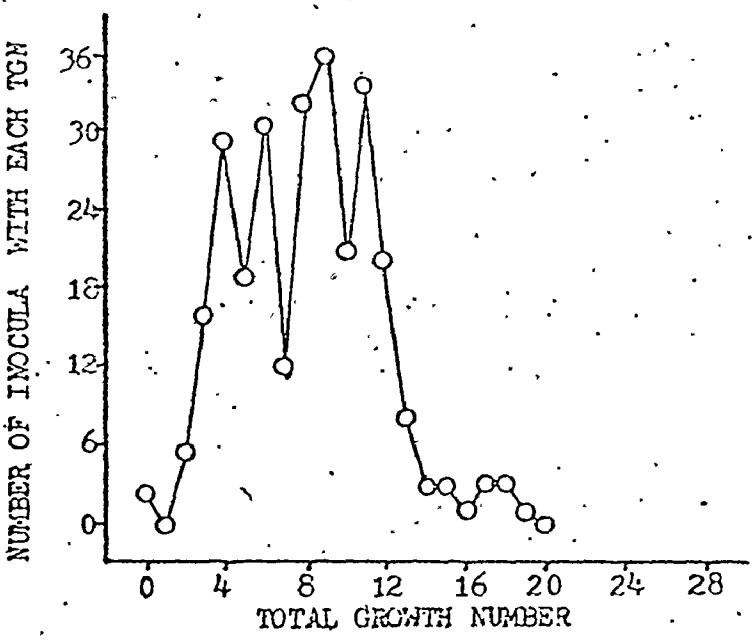
TABLE 10. One Hundred and Twenty Hour Average Counts of Conidia per Hemocytometer Field of Double Inocula Grown on Sorbose Minimal Medium at 32°C, Using Strains of the Same MT from Ascus 240. Also Including the two Parental Strains as Single Inocula .

STORE #s	Ave. Conidia per Field	(# of Trials)	STANDARD DEVIATION
1/2	13.6	(5)	5.3
1/3	9.5	(6)	1.5
1/4	8.0	(3)	1.7
2/3	5.7	(6)	3.3
2/4	13.0	(6)	6.0
3/4	3.8	(6)	1.0
5/6	346.2	(6)	33.9
5/7	332.3	(6)	54.3
5/8	203.2	(5)	32.9
6/7	419.0	(6)	52.8
6/8	202.1	(8)	94.0
7/8	212.5	(6)	10.8
PARENTS			
59-4	409.2	(5)	62.9
8-1	4.2	(6)	1.7

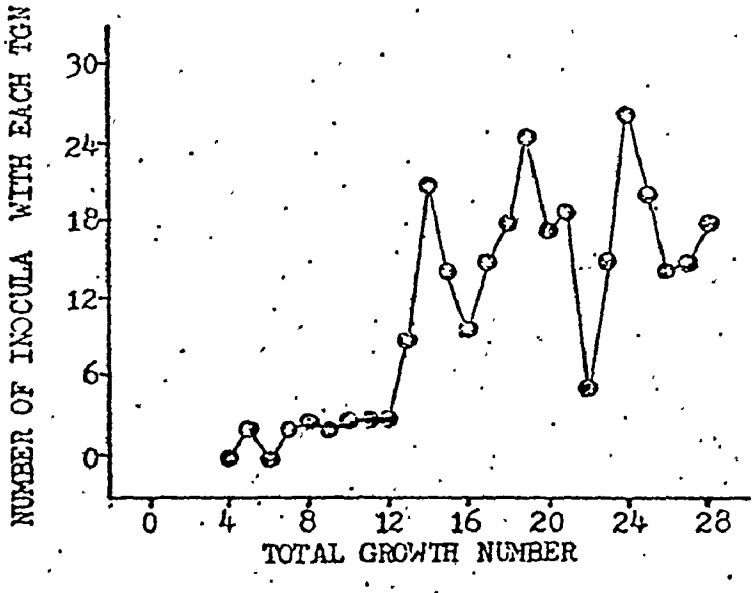
loci of major effect segregate in a cross, fewer of the progeny meet the criterion of maximum growth by 96 hrs. However, when fewer major loci were present, a more complete analysis of the nonaerial growth types could be accomplished by methods which give indications of segregation of loci of lesser effect. The segregation of loci of lesser effect could not be seen using the criterion of maximum growth by 96 hrs. After testing several different means of analysis with a desk top calculator (Materials and Methods, Section II, Part F), two methods were chosen for inclusion in a computer program for analysis of all the collected data. These two methods, from which were derived (1) total growth number (TGN) and (2) growth curve number (GCN), are presented in the Materials and Methods, Section III, and in Appendix A.

TGN values were used as a direct comparison between strains, representing both temporal and quantitative effects on aerialogenesis. These values were compared for members of spore pairs, which showed homogeneity by Student's t-test in greater than 95% of the cases at the 0.05 significance level, thus the spore pair data were combined. Values for strains were also compared to parental values before and after spore pair data were combined. These comparisons allowed for classification of strains as similar to either parent or of a nonparental type. Figure 5 illustrates the

Figure 5. Frequency distribution of TGN values for 279 inoculations each of strains 8-1(ylo$^+$, a; above) and 59-4(ylo, A; below).



O—O Strain 8-1



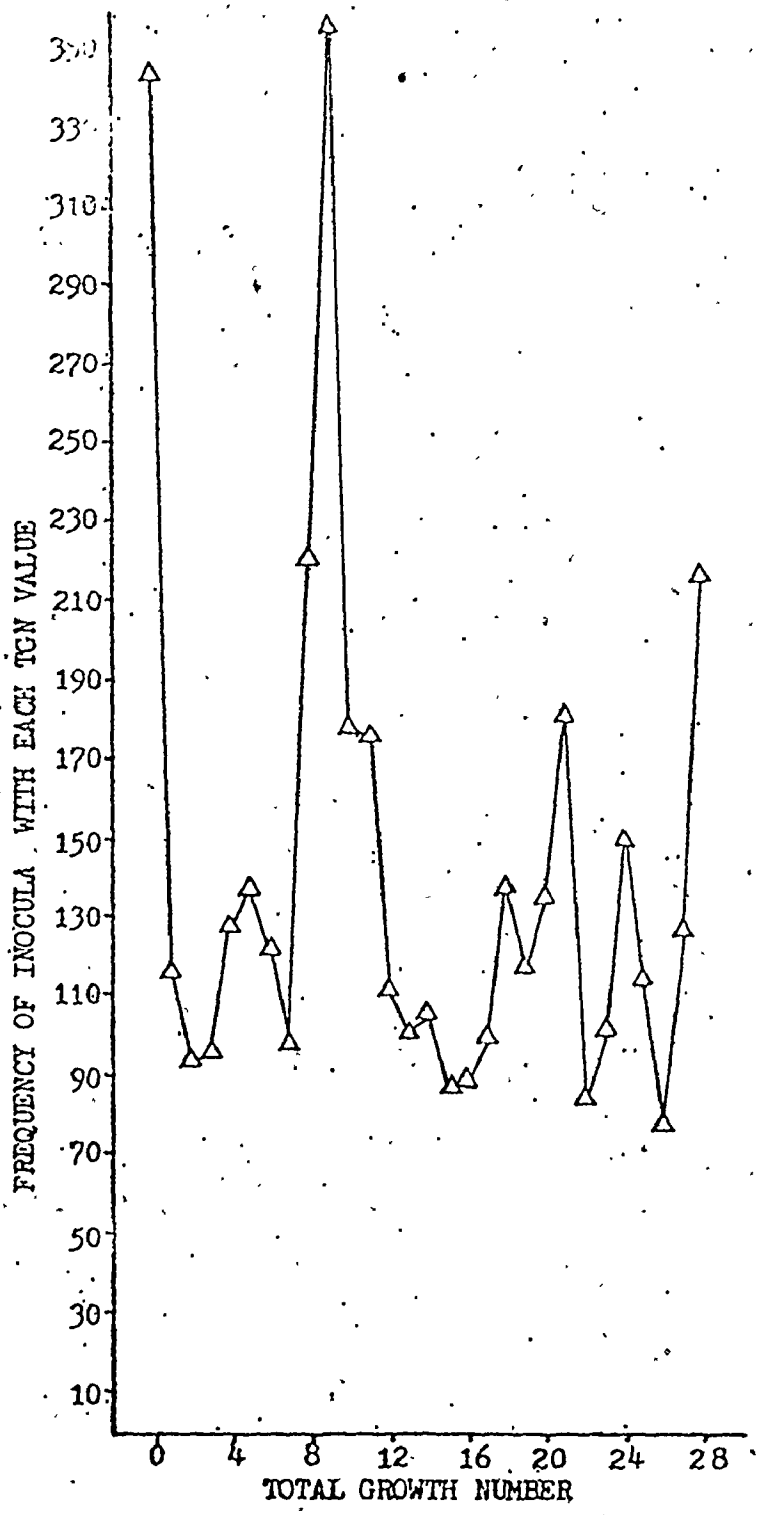
O—O Strain 59-4

distribution of TGN values for 279 platings of each of the parents in cross 7. As can be seen from this figure, there is minimal overlap between the two parental distributions. Parent 59-4 has greater than 90% of its TGN values at 14 or greater while parent 8-1 has approximately 95% of its TGN values at 13 or less. Peaks in the 8-1 parent distribution, seen in the intervals 4-6 and 8-12, should be noted and will be referred to later.

Figure 6 presents the TGN distribution for the 4,132 platings of the progeny strains from cross 7 in which the similarity of parental (Figure 5) and progeny peaks is notable. The peaks in the 4-6 and 8-12 regions, similar to the 8-1 parent are most visible. Similarities with the 59-4 parent may also be seen at TGN values of 14, 18-21 and 24 in the progeny. A large number of progeny also showed a phenotype of no aerialogenesis, giving TGN values of 0. Troughs in the distribution at 7, 15-16, 22 and perhaps 26 are also similarly represented in the parental distributions.

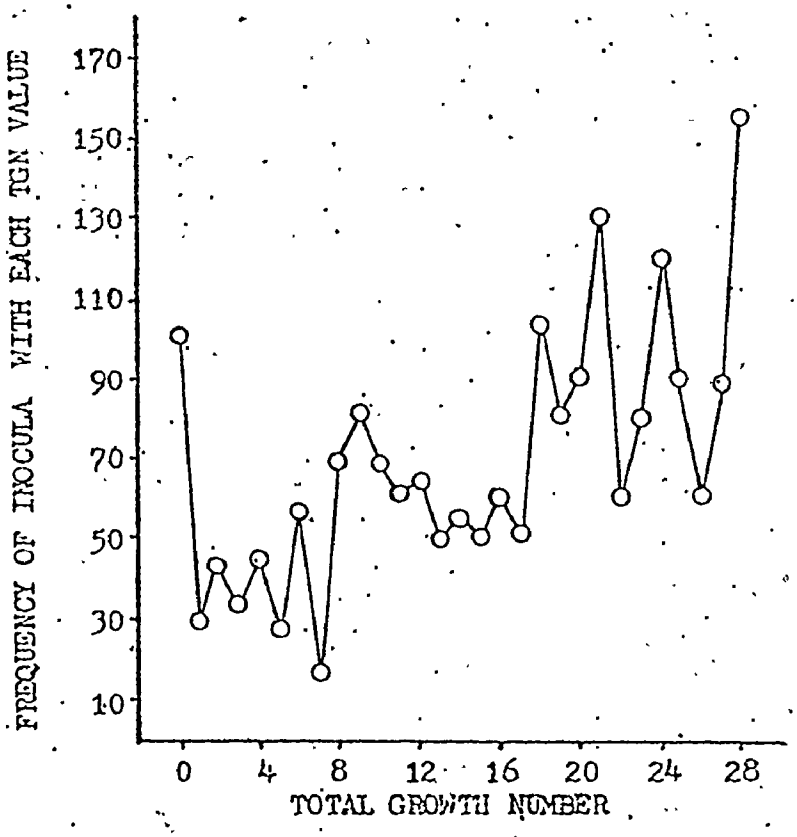
The progeny of this cross were segregating for *ylo*/*ylo*$^+$ conidia color. Figures 7 and 8 show distribution differences for aerial production between the progeny of the two conidial colors, suggesting a linkage relationship between the *ylo* locus and one or more of the aerialogenesis loci. In comparing the peaks of Figure 7 with the peaks seen for the 59-4 (*ylo*) parent (Figure 5), similarities are

Figure 6. Frequency distribution of inoculum TGNs for progeny of cross 7.



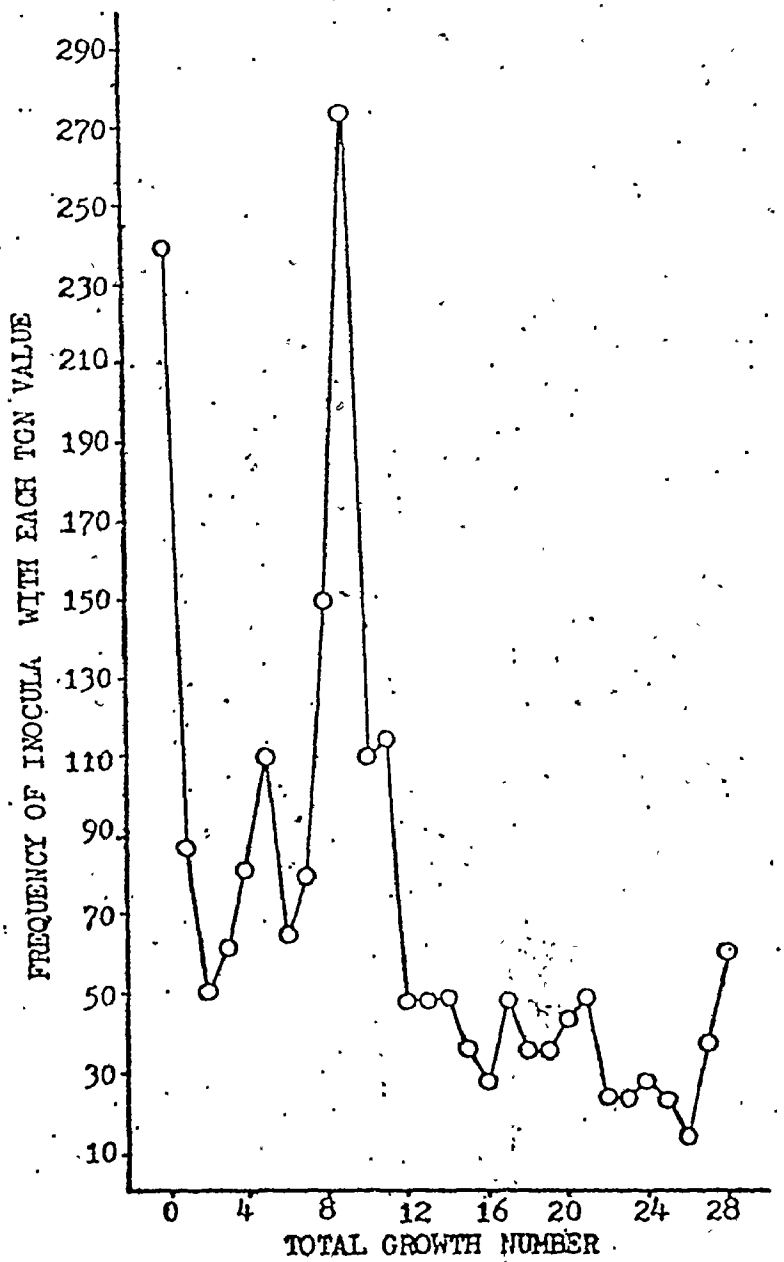
△—△ Progeny of crosses between strains 8-1 and 59-4 (4132 total inoculations)

Figure 7. Frequency distribution of inoculum TGNs for vlo progeny of cross 7.



O—O vlo progeny of crosses between strains 8-1 and 59-4 (2061 total inoculations)

Figure 8. Frequency distribution of inoculum TGNs for $\underline{vlo}^+$ progeny of cross 7.

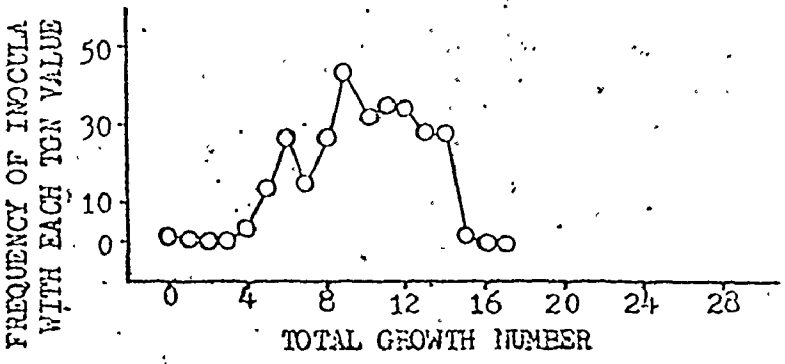


O–O $\underline{vlo}^+$ progeny of crosses between strains 8-1 and 59-4 (2071 total inoculations)

visible between 18 and 21 and at 24. Trough comparisons at 22 and 26 between these two figures also show similarities. Turning our attention to the 8-1 (*ylo*$^+$) parent, in Figure 5, and comparing it to Figure 8, the distinct peak at 9 and the smaller peak at 4-6 are evident. A notable lack of inocula showing growth greater than 13 is also visible in this same comparison. Returning to the earlier comparison it is evident that the *ylo* progeny, like the *ylo* parent, have most of the inocula with TGN values of 14 or greater.

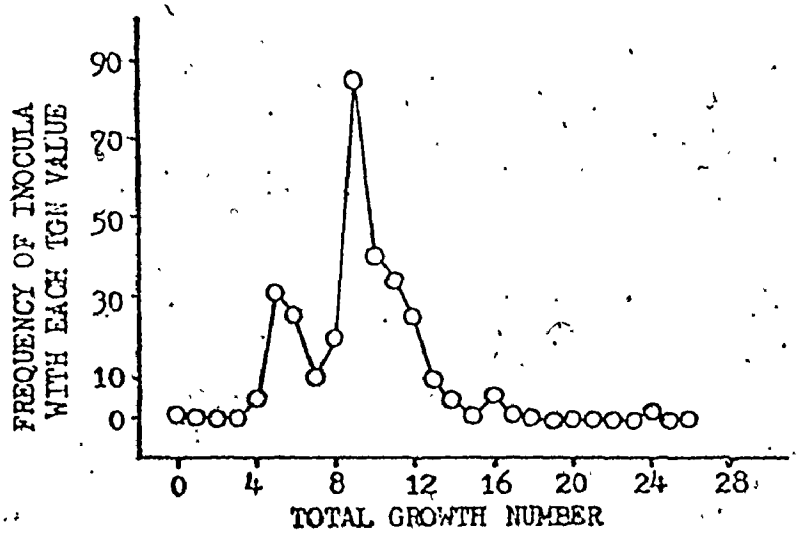
From cross 7, two asci were obtained which contained no spore pairs classifiable as aerial by the criterion of maximum growth by 96 hrs. One of these asci, number 240, was analyzed further by TGN and GCN methods with data from crosses between spores from each pair in the ascus. The TGN distributions for the four strains, one from each spore pair, are presented in Figures 9, 10, 11 and 12. Strains 240-1 and 240-3 can be seen to have peaks at 5-6 and at 9 or above, reaching a maximum at 14 (Figures 9 and 10), somewhat similar to strain 8-1 in Figure 5. Strains 240-5 and 240-7 (Figures 11 and 12) have their maximum growth peak at 4 and strain 240-7 has a large number of replicates with a TGN of 0 or 1. These parental distributions were used to differentiate progeny from the crosses into parental and nonparental types: TGN values of 1.00-5.99

Figure 9. Frequency distribution of inoculum TGNs for strain 240-1.



o—o 240-1 (*vlo*$^+$, A; 300 inoculations)

Figure 10. Frequency distribution of inoculum TGNs for strain 240-3.



o—o 240-3 (*vlo*$^+$, A; 310 inoculations)

Figure 11. Frequency distribution of inoculum TGNs for strain 240-5.

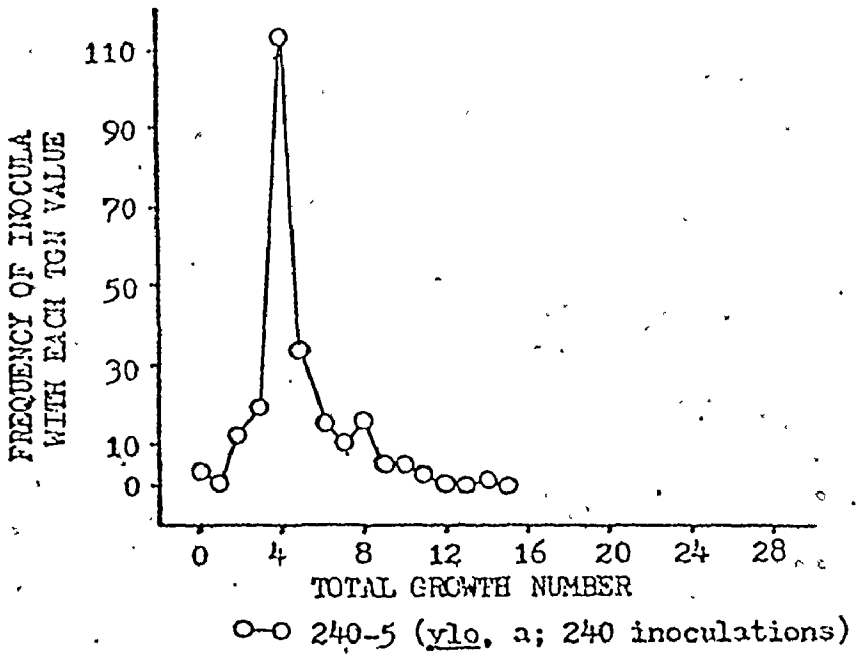
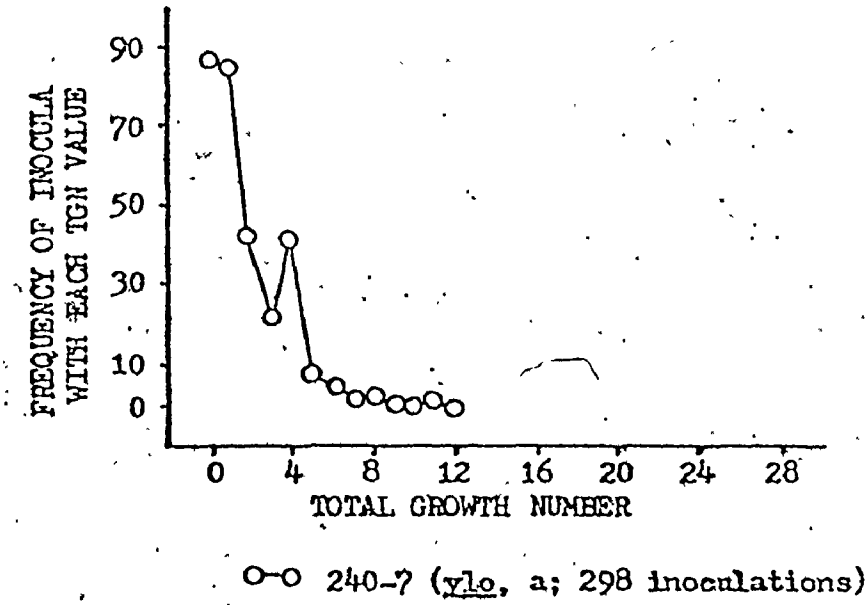


Figure 12. Frequency distribution of inoculum TGNs for strain 240-7.

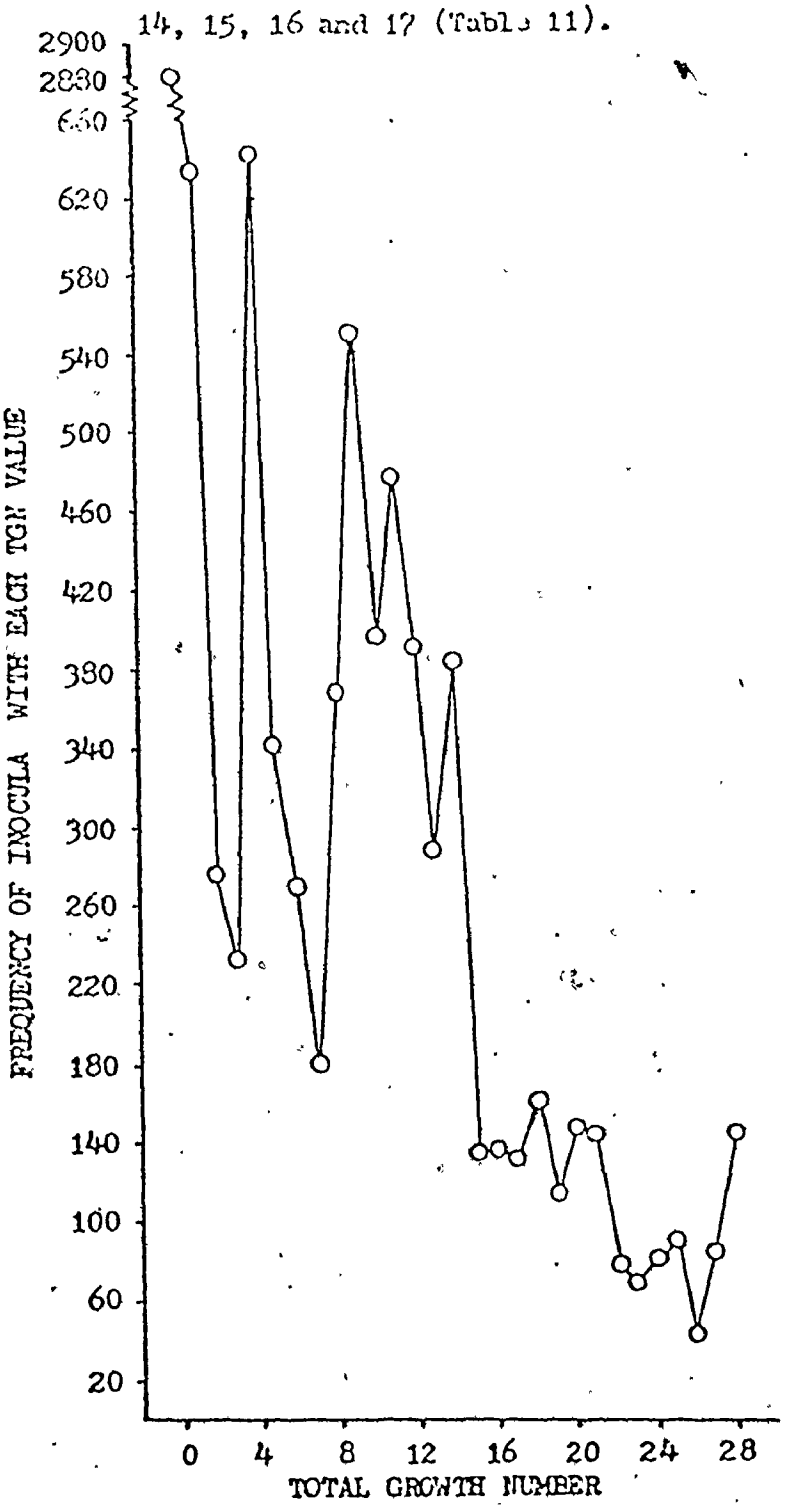


were classified as 240-5 or 240-7 parental and values of 6.00-13.99 as 240-1 or 240-3 parental. Less than 1.00 was considered as a distinct nonaerially growing (NG) phenotype and aerialogenesis with a TGN value greater than 13.99 was considered aerial in these crosses.

In Figure 13 the progeny TGN values for all crosses within ascus 240 are shown and the peaks and troughs in this distribution lend further credence to the validity of the cutoff values chosen for delineating parental and nonparental types. There is an extremely large peak at 0-1 similar to the 240-7 parent (Figure 12) and a large peak at 4 like parents 240-5 and 240-7 (Figures 11 and 12). A large proportion of the progeny are represented between 8 and 12, as is characteristic of the 240-1 and 240-3 parents (Figures 9 and 10). A trough at 13, with only a small proportion of the progeny greater than 13, would indicate a linkage relationship for at least some of the aerialogenesis loci present in this cross of two nonaerial parents.

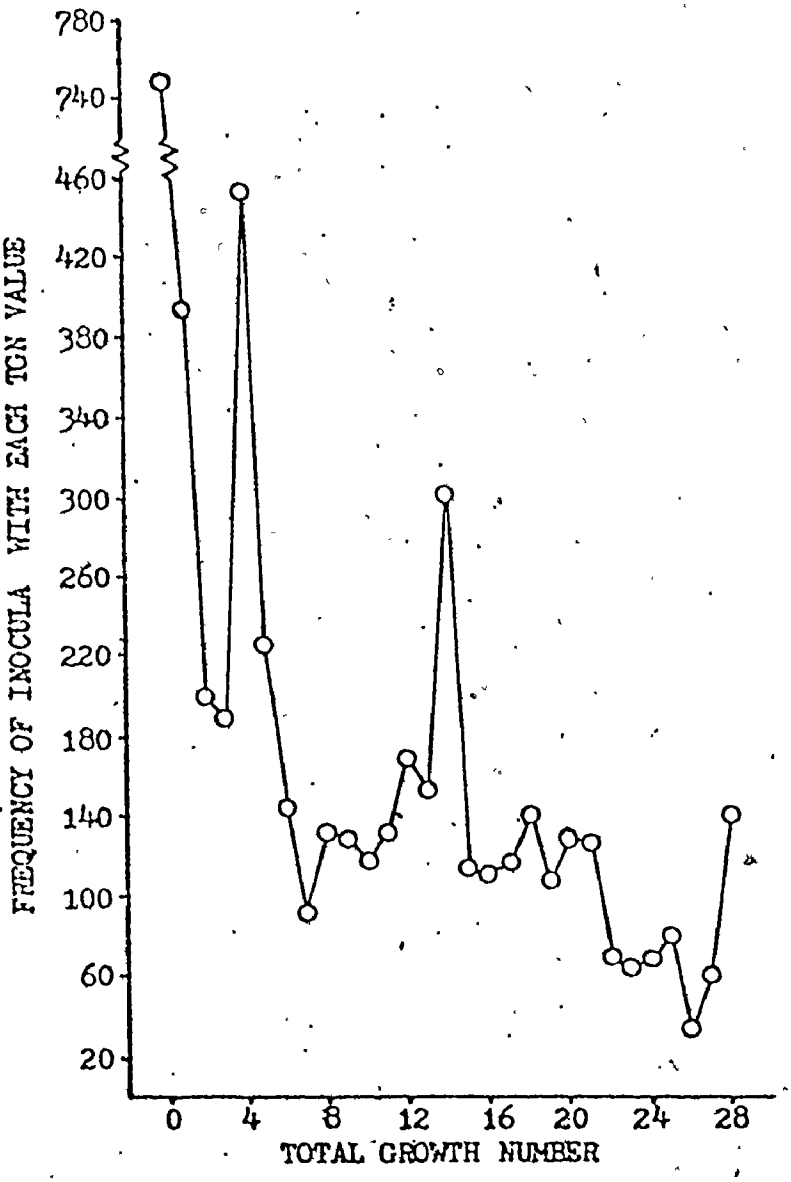
Once again, two alleles were segregating at the *ylo* locus and distributions of TGN values for progeny from crosses within ascus 240 which have been divided by conidial color, suggest linkage of one or more of the aerialogenesis loci with this marker (Figures 14 and 15). Figure 14, containing the *ylo* progeny of this cross, indicates, other

Figure 13. Frequency distribution of inoculum TGNs for progeny of crosses 14, 15, 16 and 17 (Table 11).



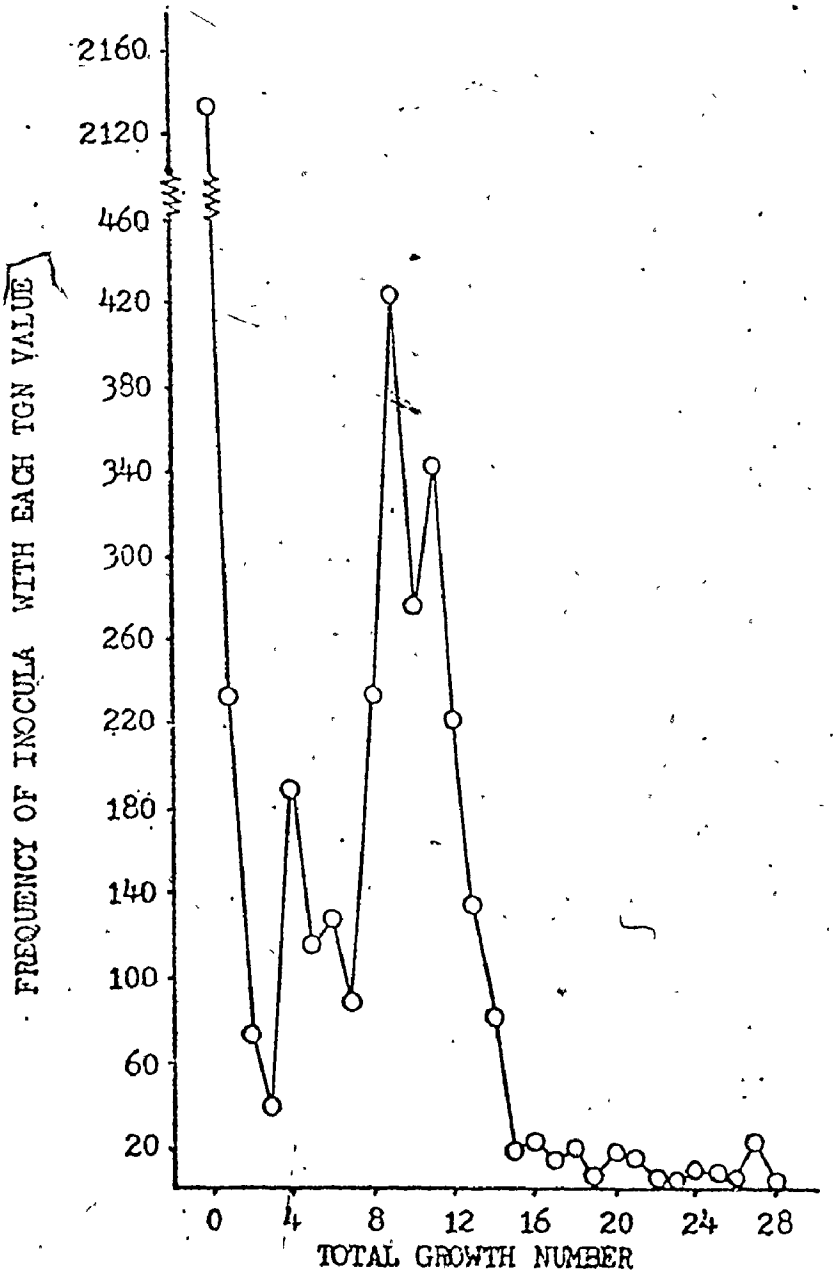
O—O Progeny of crosses between strains of opposite mt from ascus 240 (9897 inoculations)

Figure 14. Frequency distribution of inoculum TGNs for ylo progeny of crosses 14, 15, 16 and 17 (Table 11).



O—O ylo progeny of crosses between strains of opposite mt from ascus 240 (4971 inoculations)

Figure 15. Frequency distribution of inoculum TGNs for *ylo*$^+$ progeny of crosses 14, 15, 16 and 17 (Table 11).



o—o *ylo*$^+$ progeny of crosses between strains of opposite mt from ascus 240 (4926 inoculations)

than a peak at NG, a peak at 4 and 14, plus many more inocula above 14. The peak at 4 is also very characteristic of the *ylo* parents, strains 240-5 and 240-7 (Figures 11 and 12). The ylo^+ progeny, shown in Figure 15, possess the distinct peak of the ylo^+ parents; spores 240-1 and 240-3 (Figures 9 and 10), between TGN values of 8 and 12 with very few inocula giving TGN values greater than 14. Similar TGN plots were made for all of the progeny from each cross within the 240 ascus and similar results were obtained separately as those presented here in a cumulative fashion. Using these criteria, Table 11 is presented for the four crosses within the 240 ascus. The parental types and their segregation/recombination products could also be delineated using GCN values as well as TGN values, which will be discussed in the following chapter.

The analysis of data by growth curve numbers (GCNs) allowed for direct visual comparisons of the rate of aerialogenesis and the time at which it took place. GCNs were generated from the data as explained in the Materials and Methods, Section II, Part F and Section III, Part C, and Appendix A. These numbers were then used to plot growth curves for each strain tested and for the lumped spore pair data. The two parent strains were always inoculated on plates along with the progeny asci to be tested. Growth curve plots of the lumped spore pair data

TABLE 11

Asci Collected From Crosses Between Strains Within Ascus 240

aerial > 14 TGN

nonaerial < 14 TGN

#	Cross	Ascus Segregating aerial:nonaerial 4:0	3:1	2:2	1:3	0:4	Total Asci	Total % Germination
14	240-1 x 240-5		5	5	6		16	90
15	240-1 x 240-7		3	8	5		16	95
16	240-3 x 240-5		2	9	4		15	85
17	240-3 x 240-7		4	5	7		16	80

x indicates protoperithecial parent first. These crosses were attempted reciprocally but were only fertile in these configurations.

from an ascus along with the plots of the parental strains growing on the same plates were very useful in determining the phenotypes of the progeny strains and hence their proposed genotypes. Anytime there was variation in the parental growth from the norm this could be taken into consideration in the analysis of the plotted GCN values.

Figure 16 is a typical GCN plot for a first division segregation, 2:2 ascus from cross 3 (parents 8-1 and 8-5). This is a plot of ascus 210, in which the first two spore pairs had aerial growth resembling the 8-1 parent with the second two spore pairs like the 8-5 parent. The first two spore pairs also maintained the 8-1 parental conidial color phenotype of *ylo*$^+$ while the second two spore pairs were *ylo*, like the 8-5 parent. In this plot, as in all GCN plots, the time from 48 to 96 hours post inoculation is on the horizontal axis and the GCN value for the parental or combined spore pair data is on the vertical axis. The maximum GCN is 4.0 (maximum aerial growth under the experimental conditions at the time in question) and the minimum 0.0.

Figure 17 and 18 show GCN plots for typical 3:1 and 4:0 asci respectively from the same cross (cross 3, 8-1 and 8-5 parents). In these plots of ascus 152 and 182 there was first division segregation for *ylo*/*ylo*$^+$ conidial color with the first two spore pairs *ylo* in

Figure 16. GCN plot of spore pairs from ascus 210 (cross 3) with the parents, 8-1 and 8-5.

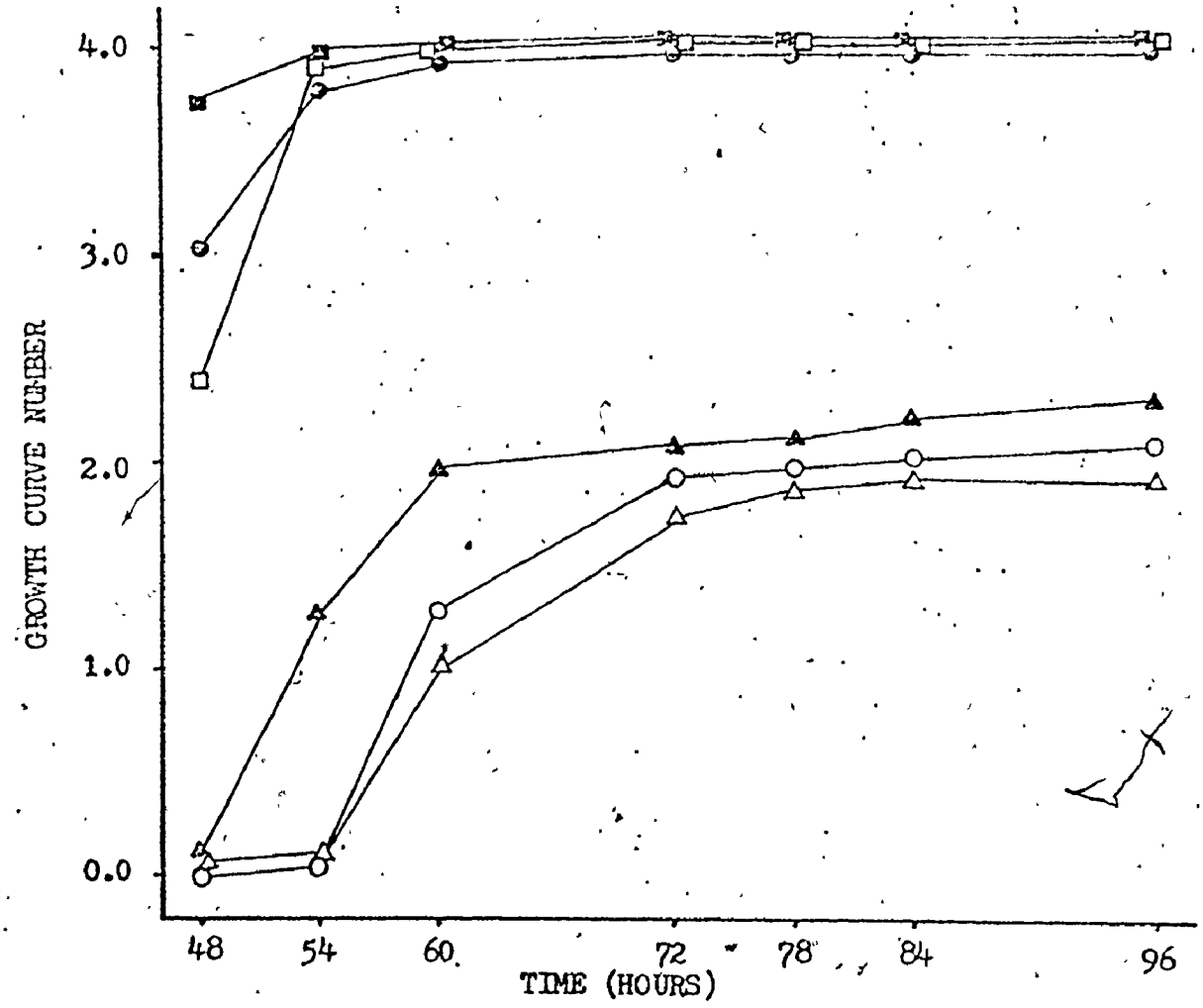


Figure 17. GCN plot of spore pairs from ascus 152 (cross 3) with the parents, 8-1 and 8-5.

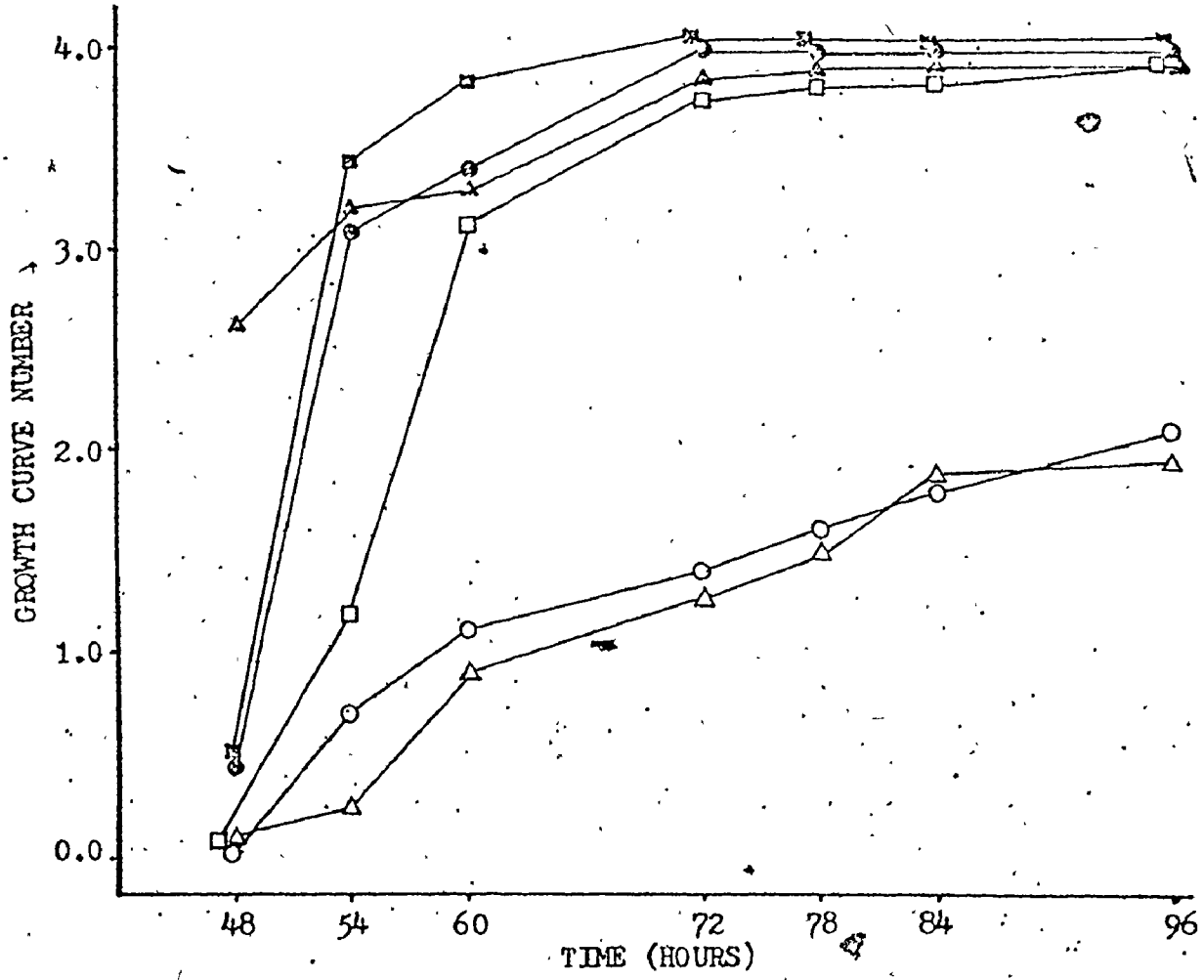
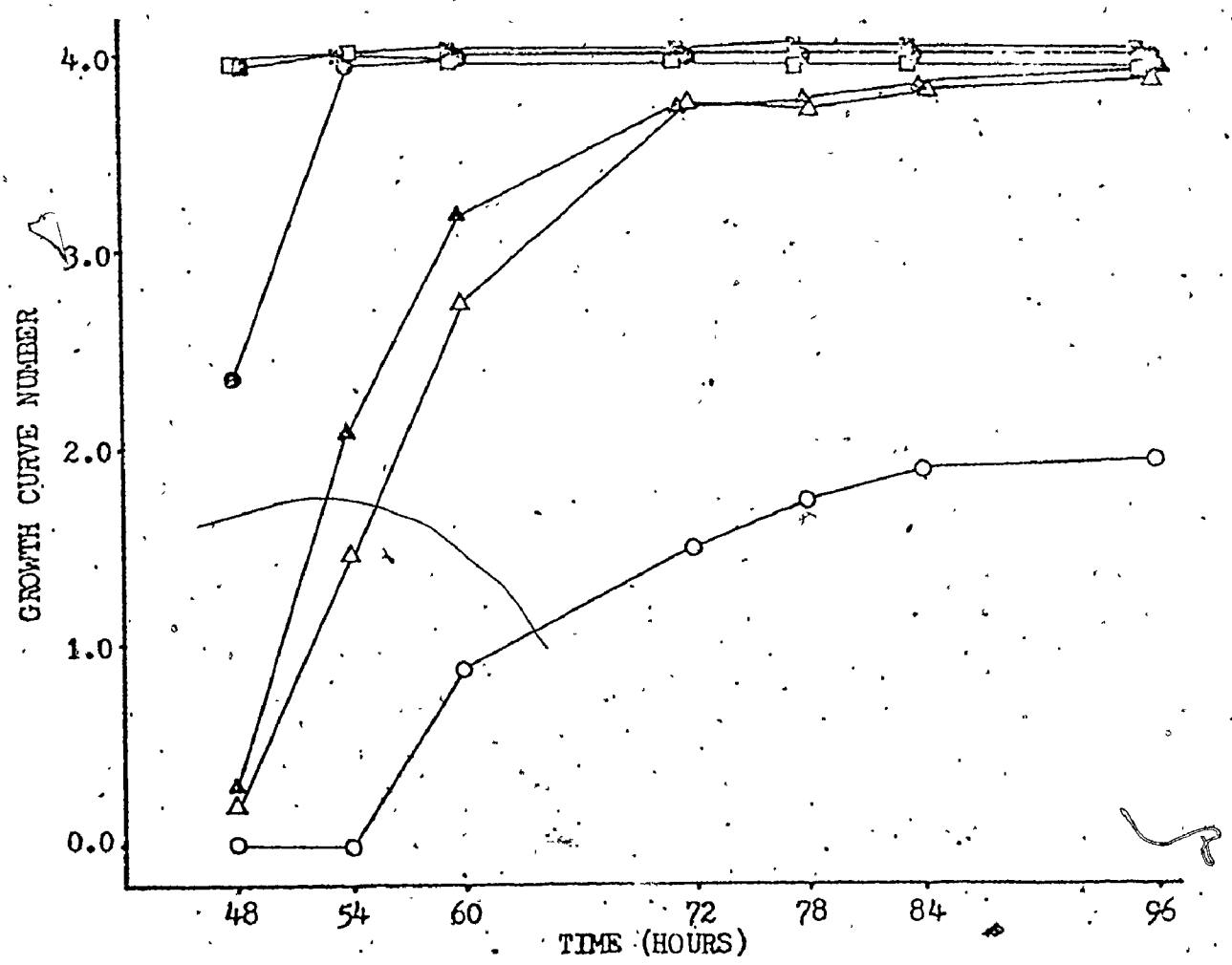


Figure 18. GCN plot of spore pairs from ascus 182 (cross 3) with the parents, 8-1 and 8-5.



ascus 152 and the second two spore pairs in ascus 182. In Figure 17 it is still quite evident that the one nonaerial spore pair is of a distinctly 8-1 parental phenotype while the three aerial spore pairs are much more similar to the 8-5 parent. In Figure 18 there were no progeny spore pairs in ascus 182 similar to the 8-1 parent with all four showing aerial growth more closely resembling the 8-5 parent.

A typical GCN plot of spore pair and parental data from cross 7 (8-1 and 59-4 parents) is shown in Figure 19 (a type II ascus, as explained on p 113f). In this plot of ascus 234 *ylo/ylo*$^+$ showed first division segregation in the same way as aerial production with the first two spore pairs being *ylo* and aerial, like the 59-4 parent, and the second two being *ylo*$^+$ and similar to the 8-1 parent in aerialogenesis. Again, a very close resemblance between progeny and parental aerialogenesis is visible.

Ascus 233, from cross 7 (parents 8-1 and 59-4), is shown in Figure 20 (a type III ascus, as explained on p 113f). This ascus shows 1:3 segregation for aerial:nonaerial phenotype and indicates a possible interaction of at least two loci in producing the maximum aerialogenesis phenotype. In this ascus there was first division segregation for *ylo/ylo*$^+$ with spore pairs three and four being *ylo*. The first spore pair exhibits a

Figure 19. GCN plot of spore pairs from ascus 234 (cross 7) with the parents; 8-1 and 59-4.

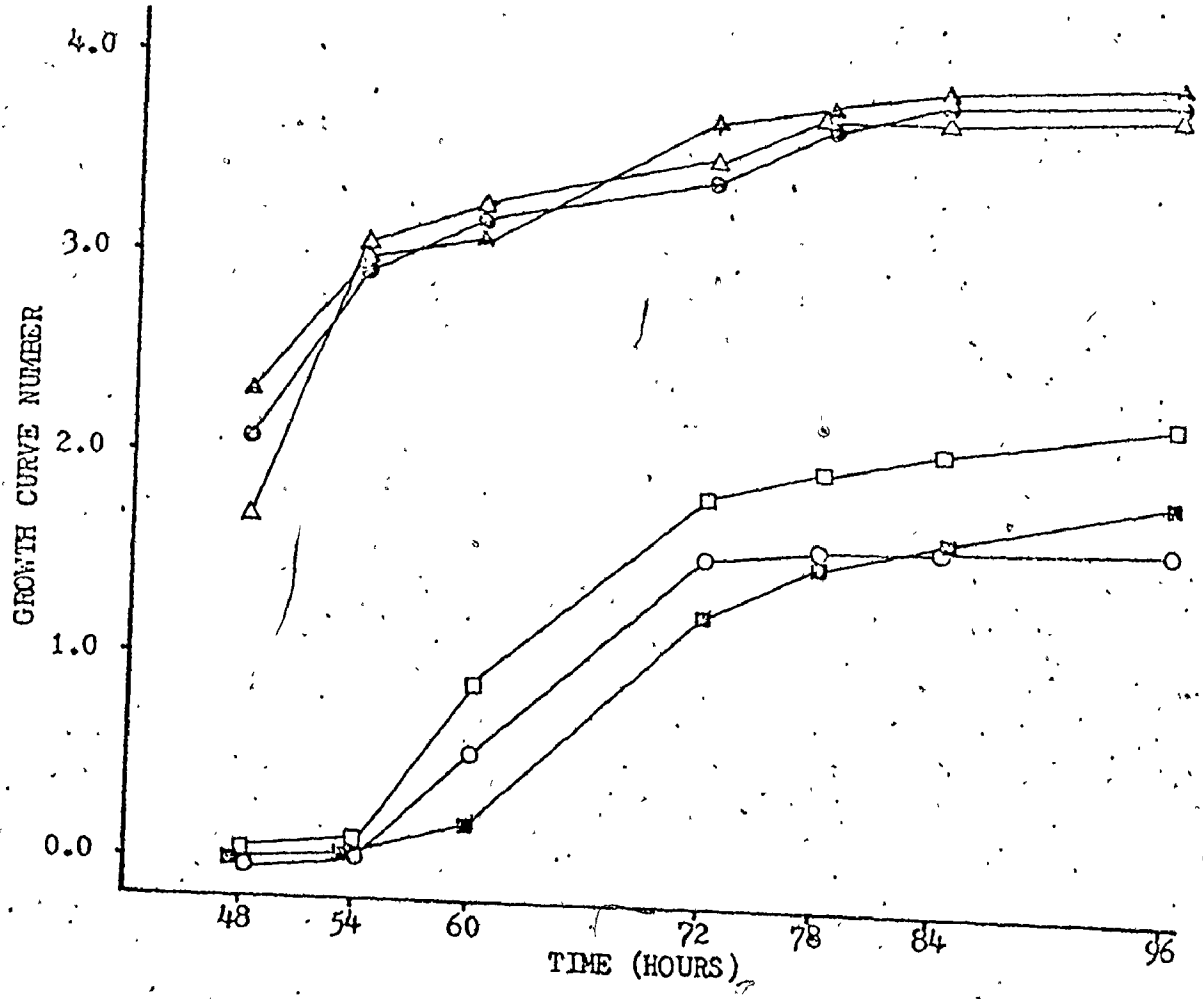
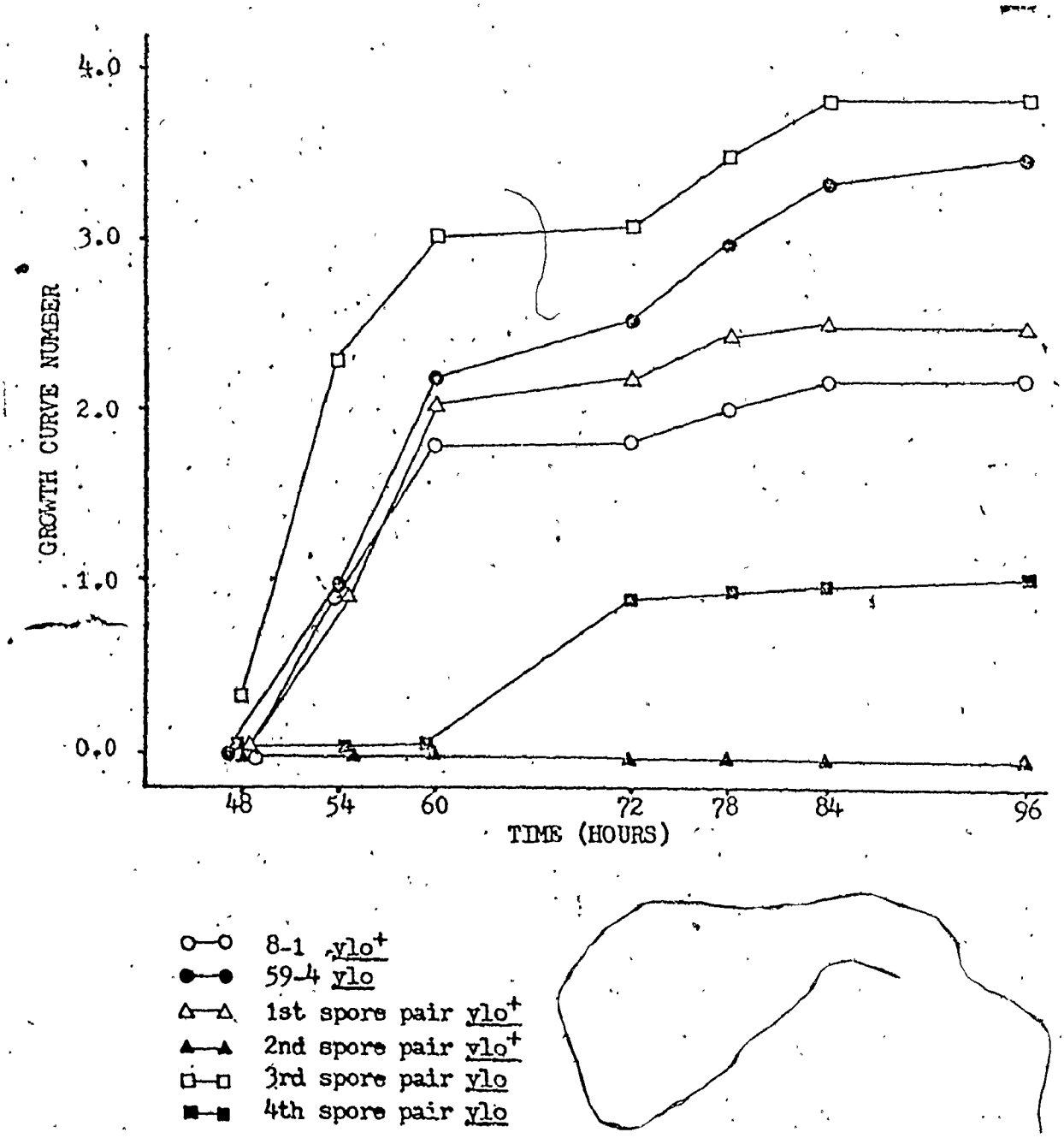


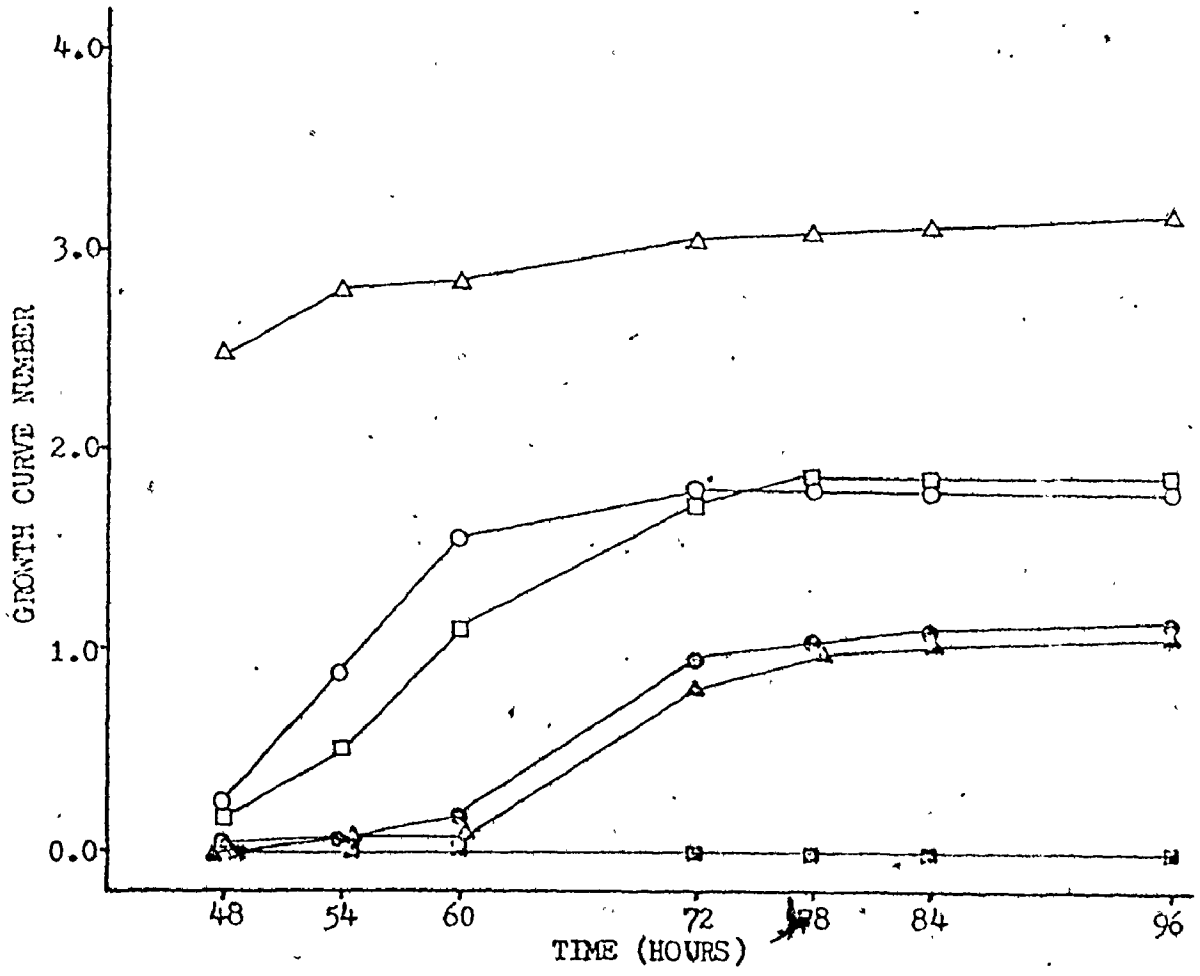
Figure 20. GCN plot of spore pairs from ascus 233 (cross 7) with the parents, 8-1 and 59-4.



phenotype similar to the 8-1 parent while pair two has a distinct nonaerial phenotype and pair three the phenotype of the 59-4 parent. Pair four has a phenotype which was not observed prior to this cross but was often observed in crosses 14 through 17 (Table 11). This phenotype is similar to that of strains 240-5, 6, 7 and 8 and is shown again in Figure 21.

From cross 7 (parents 8-1 and 59-4) two asci were obtained with no spore pairs exhibiting aerialogenesis to the extent of the aerial (59-4) parent. One of these asci, number 240, was analysed further in crosses 14 through 17 (Table 11). Figure 21 presents a typical 1:3 ascus (485) from one of these crosses (cross 16, Table 11), showing the relatively nonaerial parental growth and the one spore pair with distinctly aerial growth. This ascus showed second division segregation for *ylo*/*ylo*$^+$, with spore pairs one and three *ylo* and two and four *ylo*$^+$. The segregation of aerialogenesis phenotypes showed one spore pair similar to each parent and two dissimilar to either parent. Of the two dissimilar spore pairs, one (the fourth) was distinctly nonaerial; while the other; (the first), was distinctly aerial and much more similar to the aerial grandparent (59-4) shown in Figure 19. This also suggested that two or more loci affecting aerialogenesis segregated to give nonaerial phenotypes in ascus 240 but were available to segregate

Figure 21. GCN plot of spore pairs from ascus 485 (cross 16) with the parents, 240-3 and 240-5.



O—O 240-3 *ylo*$^+$
●—● 240-5 *ylo*
△—△ 1st spore pair *ylo*
▲—▲ 2nd spore pair *ylo*$^+$
□—□ 3rd spore pair *ylo*
■—■ 4th spore pair *ylo*$^+$

back to the original grandparental combinations in progeny of the crosses within ascus 240 (crosses 14 through 17).

Segregation patterns of the asci from the different crosses were analyzed further with these more sensitive techniques. Crosses 7, 14, 15, 16, and 17 were the only ones exhibiting the no aerial growth phenotype in the progeny. For linkage analysis, Table 12 presents ascus segregation patterns for *ylo* and aerial types from asci of crosses 1 and 8 which were second division *ylo* segregants. Crosses 3 through 6 contained no asci which were second division *ylo* segregants. Table 13 gives the ascus segregation patterns for cross 7 (parents 8-1 and 59-4) in three different groups. Type I progeny of cross 8-1 x 59-4 are presented first, followed by Type II progeny of cross 59-4 x 8-1, which showed segregation of aerial and 8-1 growth types only. Type III progeny of cross 59-4 x 8-1, which showed segregation of four aerial phenotypes on the progeny are shown in the last column. Types I and II are similar to each other and to the phenotypes observed in all the previous crosses. Type III is distinctly different from all previous progeny, including aerial and 8-1 growth types observed before, along with a growth type similar to strains 240-5 and 7, which were later used as parents, and a nonaerial growing type (NG). The overall segregants from Table 13 contain 33 *ylo* aerial

TABLE 12

Asci From Crosses 1 and 8 Which are Second Division Segregants for *ylo*/*ylo*$^+$.

Cross 1

Ascus #	11	161	168
	ylo 8-1	*ylo*$^+$ aerial	*ylo*$^+$ 8-1
	ylo$^+$ aerial	*ylo* aerial	*ylo* aerial
	ylo aerial	*ylo*$^+$ 8-1	*ylo* 8-1
	ylo$^+$ aerial	*ylo* aerial	*ylo*$^+$ aerial

Cross 8

Ascus #	311
	ylo aerial
	ylo$^+$ aerial
	ylo$^+$ aerial
	ylo aerial

Crosses 3-6 contain no asci which are second division segregants for *ylo*/*ylo*$^+$.

TABLE 13

Ascus Segregation Patterns From Cross 7, 8-1 x 59-4.

Type I			
	ylo aerial	*ylo* 8-1	*ylo* 8-1
	ylo aerial	*ylo* aerial	*ylo* 8-1
	ylo$^+$ 8-1	*ylo*$^+$ aerial	*ylo*$^+$ aerial
	ylo$^+$ 8-1	*ylo*$^+$ 8-1	*ylo*$^+$ aerial
ascus #	254,504,249, 253	501,503	247
	ylo aerial		
	ylo$^+$ aerial		
	ylo 8-1		
	ylo$^+$ 8-1		
	250		

asci isolated on 2 separate occasions from 2 different replicates of this cross.

Ascus Segregation Patterns From Cross 7, 59-4 x 8-1.

Type II		Type III	
ylo 1-3	269	*ylo*$^+$ 5-7	273
ylo aerial	230	*ylo*$^+$ 1-3	
ylo$^+$ aerial		*ylo* NG	
ylo$^+$ 1-3		*ylo* aerial	

TABLE 13 (continued)

ylo aerial	270,235	*ylo*$^+$ 1-3	233
ylo aerial	271,241	*ylo*$^+$ NG	
ylo$^+$ 1-3	274	*ylo* aerial	
ylo$^+$ 1-3	234	*ylo* 5-7	
ylo aerial	275	*ylo* aerial	237
ylo$^+$ aerial		*ylo* aerial	238
ylo$^+$ 1-3		*ylo*$^+$ NG	
ylo 1-3		*ylo*$^+$ NG	
		ylo$^+$ 1-3	240
		ylo$^+$ 1-3	272
		ylo 5-7	
		ylo 5-7	
ylo aerial	268	*ylo* 1-3	278
ylo 8-1		*ylo*$^+$ 5-7	
ylo$^+$ 8-1		*ylo* NG	
ylo$^+$ 8-1		*ylo*$^+$ aerial	
		ylo NG	239
		ylo aerial	
		ylo$^+$ 1-3	
		ylo$^+$ NG	

5-7 indicates growth similar to that found in ascus 240, spores 5 and 7.
NG indicates no aerial growth
all asci isolated on same day from same cross
1-3 indicates growth similar to that found in asci 240, spores 1 and 3

spore pairs, 19 *ylo* nonaerial spore pairs and 11 and 41 aerial and nonaerial *ylo*$^+$ spore pairs respectively. Once again a linkage relationship to *ylo* is suggested although more different phenotypes may now be defined.

Using this more sensitive analysis, the aerial growth phenotypes of the progeny were determined for all asci from crosses 14 through 17. These data are presented in Tables 14 through 17 as ascus segregation patterns from each of these crosses between strains from ascus 240. Aerial refers to spore pairs with a TGN value greater than 13.99 and a plotted GCN curve similar to the 59-4 grandparent. NG refers to spore pairs which had little or no aerial growth, a TGN of 0.99 or less, and an essentially horizontal GCN plot from the point of origin. The 1-3 phenotype is similar to that observed in the relatively nonaerial grandparent strain (8-1) which has now been defined as having a TGN between 6.00 and 13.99. Strains 240-1 and 3 have the 8-1 phenotype and a characteristic GCN plot for any one set of replicates can be compared to the progeny strains tested at the same time. A TGN value between 1.00 and 5.99 and a GCN plot similar to spores 240-5 or 7 indicates a growth phenotype classified as 5-7.

TABLE 14

Ascus Segregation Patterns From Cross 14, 240-1 x 240-5.

ylo 5-7	*ylo* aerial	ylo^+ 5-7
ylo 5-7	*ylo* aerial	ylo^+ 1-3
ylo^+ 1-3	ylo^+ NG	*ylo* aerial
ylo^+ 1-3	ylo^+ NG	*ylo* NG
472,469,471,653	461,470,651,654	467
ylo^+ NG	ylo^+ 1-3	*ylo* 1-3
ylo^+ 1-3	ylo^+ aerial	ylo^+ 1-3
ylo 5-7	*ylo* 5-7	ylo^+ 5-7
ylo aerial	*ylo* NG	*ylo* 5-7
473	465	466
ylo aerial	*ylo* aerial	ylo^+ NG
ylo^+ 5-7	ylo^+ aerial	ylo^+ 1-3
ylo^+ 1-3	ylo^+ NG	*ylo* 1-3
ylo NG	*ylo* 5-7	*ylo* 5-7
464	463	462
ylo aerial		
ylo NG		
ylo^+ NG		
ylo^+ 1-3		
655		

TABLE 15

Ascus Segregation Patterns From Cross 15, 240-1 x 240-7.

ylo 5-7	*ylo* aerial	*ylo* aerial
ylo 5-7	*ylo* 5-7	*ylo* aerial
ylo$^+$ 1-3	*ylo*$^+$ NG	*ylo*$^+$ NG
ylo$^+$ 1-3	*ylo*$^+$ 1-3	*ylo*$^+$ NG
455,459,606	448,452,460,602,599	451,453,603
ylo$^+$ 5-7	*ylo* 5-7	*ylo* NG
ylo NG	*ylo* NG	*ylo* 5-7
ylo$^+$ 1-3	*ylo*$^+$ 1-3	*ylo*$^+$ NG
ylo aerial	*ylo*$^+$ aerial	*ylo*$^+$ 1-3
454	605	601
ylo 1-3 (aerial?)	*ylo* 5-7	
ylo$^+$ NG	*ylo* aerial	
ylo NG (5-7?)	*ylo*$^+$ NG	
ylo$^+$ NG	*ylo*$^+$ NG	
604	607	

TABLE I6

Ascus Segregation Patterns From Cross 16, 240-3 x 240-5.

ylo 5-7	*ylo* aerial	ylo^+ NG
ylo 5-7	*ylo* 5-7	ylo^+ NG
ylo^+ 1-3	ylo^+ 1-3	*ylo* aerial
ylo^+ 1-3	ylo^+ NG	*ylo* aerial
645	477,478,484,647	483,640
ylo^+ 1-3	*ylo* aerial	*ylo* aerial
ylo^+ 5-7	ylo^+ 5-7	*ylo* NG
ylo aerial	*ylo* 1-3	ylo^+ NG
ylo NG	ylo^+ NG	ylo^+ NG
481	485	637
ylo^+ NG	ylo^+ NG	*ylo* NG
ylo^+ NG	ylo^+ NG	*ylo* NG
ylo 5-7	*ylo* 5-7	ylo^+ 1-3
ylo 5-7	*ylo* aerial	ylo^+ 1-3
638	641	642
ylo^+ 1-3	*ylo* NG	
ylo^+ NG	*ylo* aerial	
ylo NG	ylo^+ NG	
ylo 5-7	ylo^+ NG	
646	649	

TABLE 17

Ascus Segregation Patterns From Cross 17, 240-3 x 240-7.

ylo 5-7	*ylo* aerial	*ylo* 1-3
ylo 5-7	*ylo* aerial	*ylo* 5-7
ylo$^+$ 1-3	*ylo*$^+$ NG	*ylo*$^+$ 5-7
ylo$^+$ 1-3	*ylo*$^+$ NG	*ylo*$^+$ 1-3
487,493,619,620	617,625,623,624	495,618

ylo NG	*ylo* 5-7	*ylo*$^+$ 5-7
ylo aerial	*ylo* NG	*ylo*$^+$ NG
ylo$^+$ 1-3	*ylo*$^+$ aerial	*ylo* 1-3
ylo$^+$ 5-7	*ylo*$^+$ 1-3	*ylo* aerial
614,626	621	490

ylo 5-7	*ylo* NG
ylo aerial	*ylo* 5-7
ylo$^+$ 1-3	*ylo*$^+$ 1-3
ylo$^+$ NG	*ylo*$^+$ 1-3
615	622

DISCUSSION

Certain conclusions may be drawn concerning the aerialogenesis loci segregating in the *Neurospora crassa* strains used for this study. These conclusions are summarized here, followed by a discussion of their implications and the basis for their validity.

1.) There is a single locus segregating two alleles, one of which is associated with maximum aerialogenesis and one with demonstrably less aerialogenesis.
   a) This locus is referred to as *aer-1*.
   b) This locus segregates independently from the mt locus on LG I.
   c) This locus also segregates independently from the *ylo* locus on LG VI.
   d) This locus has no apparent quantitative or qualitative effects on conidiogenesis as far as they can be separated from aerialogenesis.

2.) There are two other loci segregating in the original crosses which are able to interact in one allelic combination to give maximum aerialogenesis.
   a) These two loci have been designated *aer-2* and *aer-3*.
   b) These two loci segregate in a linked fashion with

the *ylo* locus on LG VI.

c) These two loci are closely linked on the right arm of LG VI while the *ylo* locus is proximal on the left arm.

d) These loci have no apparent qualitative effect on conidiogenesis but are found associated with a distinct quantitative effect.

3.) During the course of this study at least one new allele arose which has a quantifiable effect on aerialogenesis.

a) This locus was designated *aer-3* and the new allele *aer-3*$^{+}$.

b) The *aer-3*$^{+}$ allele is associated with a distinct nonaerial growth (NG) phenotype, exhibited by some spore pairs, and a general reduction in aerialogenesis by those strains hypothesized to contain the *aer-3*$^{+}$ allele.

c) The *aer-3* allele is able to interact with the aerialogenesis loci on LG VI (*aer-1* and *aer-2*) to give maximum aerialogenesis.

d) This locus is unlinked to the *ylo* locus and the 2 aerialogenesis loci (*aer-1* and *aer-2*) on LG VI.

e) The locus is also unlinked to the *mt* locus on LG I.

4.) Statistically significant homogeneity was found between spore pairs throughout the study.

a) This homogeneity, observed for genetically identical strains, is indicative of the usefulness of the new techniques developed during this study for the analysis of aerialogenesis.

b) The observed homogeneity between spore pairs also indicates that, in these strains, any contribution of cytoplasmic variation within an ascus to the aerialogenesis variation is minor.

5.) Aerialogenesis in *Neurospora crassa* is under genetic control by several loci and the contribution of an allele may be quantified by the techniques described.

6.) In the asci tested, variation in conidiogenesis segregated as would be expected for a nuclear locus.

7.) Conidiogenesis and aerialogenesis segregate as genetically determined separable events with no consistent quantitative correlation between them.

8.) No genetic effects were seen on the qualitative aspects of conidiogenesis although such effects have been reported previously (Barratt and Garnjobst, 1949; Grigg, 1958, 1960a and b).

From the ascus segregation patterns observed in cross 1 (Table 5 in the Results Chapter), it is evident

that there are 2 independent means of generating maximum aerialogenesis segregating in the parental strains. If no segregation had been observed, then the conclusion would be that aerialogenesis in the parents was produced by the same genetic means and hence all the progeny were also aerial. The appearance of 3:1 and 2:2 segregation patterns in the progeny asci from this cross indicates that at least 2 independent means for producing maximum aerialogenesis must be segregating. In cross 2, the absence of segregation indicates that the parental strains have a genetic capability for maximum aerialogenesis in common and hence all the progeny are like the parents. In crosses 3 through 6 (Table 5), each involving the cross of a nonaerial by an aerial parent derived from the same 2:2 ascus, the segregation patterns again indicate two separable means of producing maximum aerialogenesis. Thus, any explanations of the aerialogenesis capabilities segregating in these crosses must include sufficient loci to produce maximum aerialogenesis via at least two independent and nonoverlapping allelic combinations. In ascus 8 there was first division segregation for the *ylo* locus, and each of the two allelic combinations for producing maximum aerialogenesis. This led to an ascus with the first two spore pairs

exhibiting *ylo*$^+$ conidial color and the nonaerial phenotype which has since been designated 8-1, after the phenotype of that strain. The second two spore pairs have *ylo* conidial color and the maximum aerial growth phenotype.

Ascus 59 offered an opportunity to separately analyze the two genotypes for maximum aerialogenesis. In this 3:1 ascus four genotypes were present which led to the segregation pattern of 3 spore pairs giving maximum aerialogenesis and 1 spore pair being of the 8-1 nonaerial phenotype. Of the three aerial pairs, the first possessed both capabilities of producing maximum aerial growth as shown by the 4:0, 3:1 and 2:2 segregation patterns observed in the progeny asci from the cross of 59-1 with 8-1 (Cross 8, Table 5 in the Results Chapter). The second pair possessed only one genotype for producing maximum aerialogenesis and this genotype appeared to be made up of two loci separable by recombination or segregation. This is evident from the 2:2, 1:3 and 0:4 segregation patterns (Cross 7, Table 5). Only 2:2 segregation would be expected if a single locus was contributing to this capability of producing maximum aerialogenesis. Much more will be said about this cross and the progeny produced later in the discussion when the analysis employs more sensitive techniques than merely aerial vs. nonaerial in growth type. The third spore pair is itself nonaerial and

when crossed with another nonaerial strain gives 0:4 segregation patterns throughout (Cross 10, Table 5 in the Results). This is a good indication that the third spore pair contains no alleles involved in the production of maximum aerialogenesis. In the first three spore pairs, three of the four genotypes for producing maximum aerialogenesis expected to be present in the progeny ascus, have been found and in the fourth spore pair the segregation pattern of 2:2 in all the progeny asci indicates one more segregating genotype for production of maximum aerialogenesis (Cross 9, Table 5 in the Results). The last spore pair contains one locus which is able to provide maximum aerialogenesis and does not break down in the progeny due to recombination or segregation, as the locus in the second spore pair was observed to do.

The single locus present in the fourth spore pair of ascus 59 (cross 9) was analyzed for linkage relationships with other known markers present in the strain. From Table 6 (in the Results Chapter), it is apparent that this locus is segregating independently from the *ylo* locus on LG VI. Similar numbers of PD and NPD asci indicate that these loci are unlinked while large numbers of T asci indicate that the locus must be distal on its chromosome (the *ylo* locus was previously shown to be proximal on LG VI and this position was

substantiated throughout these experiments). Similar analysis indicated independent segregation with respect to the mating type (*mt*) locus on LG I. This single locus associated with maximum aerialogenesis, is designated *aer-4* and its wild type allele, which does not contribute to maximum aerialogenesis, as *aer-4*$^{+}$.

Crocken and Tatum (1967) stated that wild type *Neurospora*, grown on minimal medium supplemented with 0.2% glucose and 2.0% sorbose, grow colonially, fail to conidiate, and lose up to 60% of their dry weight after the glucose is depleted in the medium. Hence, any of the strains used in this study which appear to undergo aerial differentiation, in spite of the presence of 1% sorbose, were defined as containing the mutant allele while their counterpart strains which did not undergo aerialogenesis under the experimental conditions have been referred to as wild type and given the "+" designation. In all cases, the strains used in this study maintained a colonial morphology, in terms of colony diameter on the agar surface, and the only variation was that found in aerialogenesis phenotype. From Tables 8 and 9 (in the Results Chapter) there is no apparent qualitative or quantitative effect of this locus on conidiogenesis under these experimental conditions. In Table 8, strain 8-1 contains the *aer-4*$^{+}$ allele and

strain 8-5 the *aer-4* mutant allele. There were no consistent differences noted between these two strains in the qualitative aspects of conidiogenesis based on the variables of time, temperature, medium composition, or strain (genotype). The first and fourth spore pairs of ascus 59 contain the *aer-4* allele while the second and third spore pairs had the *aer-4*$^{+}$ allele. In Table 9 a first division segregation for the quantitative aspects of conidiogenesis can be seen while the *aer-4* locus has undergone second division segregation, indicating that these are two genetically separable events. Table 6 also shows the results obtained from cross 12 (parents 131-7 and 8-1) in respect to the segregation of the *ylo* locus and a single locus able to produce maximum aerialogenesis *(aer-4)*. The cross of strains 59-8 and 275-3 (cross 9) gave one PD, no NPD, and thirteen T asci while the cross of strains 131-7 and 8-1 (cross 12) gave no PD, one NPD and nine T asci. These two sets of data are in very close agreement and it is proposed that strains 59-8 and 131-7 each contain the *aer-4* allele and that this locus is distal on its chromosome. The approximate distance of the *aer-4* locus from its centromere may be calculated using the formula:

$$\frac{2^{ND} \text{ division segregants}}{\text{total asci}} \times 2$$

hence, $\frac{21}{24} \times 2$ giving 43.75% recombination.

The results are most consistent with the hypothesis that the *aer-4* locus and the other maximum aerialogenesis genotype are segregating in crosses 1 through 6 and 8 while *aer-4* is the only maximum aerialogenesis genotype segregating in crosses 9 and 12.

Asci collected from crosses between strains 8-1 and 59-4 (cross 7) have been divided into three types based on the female parent for the cross and the aerialogenesis phenotypes exhibited by the progeny (Table 13). Type I asci are derived from the cross using strain 8-1 as the protoperithecial parent. These eight asci contain strains exhibiting the two phenotypes observed in all previous crosses, the 8-1 relatively nonaerial type, and the maximum aerialogenesis phenotype. All of the asci were 2:2 aerial:nonaerial with two of the eight asci being second division aerial. The two second division aerial asci were first division for *ylo* and the one ascus which was second division for *ylo* was a first division aerial segregant. This indicates that if two

loci are interacting to produce the aerial phenotype in strain 59-4, they are very closely linked. The maintenance of the parental combination of *ylo* and aerial indicates that the two are on the same linkage group. The segregation analysis indicates that the *ylo* locus is on the opposite side of the centromere from the proposed two closely linked loci which interact to produce the maximum aerialogenesis phenotype.

Type II and III asci were isolated from the same cross tube on the same day (Table 13). Strain 59-4 was used as the protoperithecial parent in this cross with strain 8-1. When analyzed by means of the aerial: nonaerial numbers by 96 hrs these asci appear as one class of progeny and were represented as such in Table 5. However, when these eighteen asci are subjected to more sensitive tests using TGN and GCN techniques it becomes apparent that there are two distinct types of asci being produced by this single cross. Strains from Type II asci possess the same two phenotypes found in Type I asci and these ten strains approximate the same segregation patterns. Once again, there are two asci which are second division segregants for aerial and first for *ylo* while there is one ascus exhibiting first division segregation for aerial and second for *ylo*. This agrees with the previous hypothesis that the aerialogenesis

phenotype is linked to *ylo* but on the opposite side of the centromere, when considered with the observation that there were no asci which were second division segregants for both *ylo* and the aerialogenesis phenotype in either Type I or II asci. The Type II class contains one ascus in which recombination appears to have occurred between the two interacting loci in strain 59-4, producing a 1:3 segregation pattern with three nonaerial spore pairs of the 8-1 parental phenotype. The eight asci grouped in the Type III class all possess at least one spore pair with a nonaerial phenotype unlike the 8-1 parent. Three nonaerial phenotypes were observed in these asci; the aforementioned 8-1 type, a nonaerial growing type (NG), and a type intermediate between these two which was exemplified by strains 240-5 and 240-7, hence the phenotypic symbol 5-7. Figures 20 and 21 in the Results Chapter illustrate the GCN plots of these three relatively nonaerial growth types. Type III asci also contain maximum aerialogenesis strains similar in phenotype to the 59-4 parent.

Analysis has shown that the maximum aerialogenesis phenotype from the *ylo* parent (59-4) is maintained in the progeny of Types I and II in a way which indicates linkage of the *ylo* and aerialogenesis loci. The Type III asci suggest a very loosely linked or unlinked condition

for these two phenotypes and hence their aerialogenesis loci. The crosses between strains of ascus 240 also indicate that the maximum aerialogenesis phenotype is unlinked to the *ylo* phenotype (Tables 14-17). In these four tables (14 through 17), one for each of the four crosses between strains representing each of the four spore pairs of ascus 240, there were twelve PD asci and thirteen NPD asci in terms of conidial color and aerialogenesis phenotypes. In all previous crosses it appeared that if two loci were interacting to produce the maximum aerialogenesis phenotype seen in strain 59-4, then they must be closely linked to each other and also linked to the *ylo* locus. From this anomaly it seems most probable that a new mutation or reversion has occurred during the course of the crossing and testing of these strains. If this is true, and the new allele is recessive to the allele originally found in these stocks, then the effect of the allele might not be observed until segregation for it was observed in the progeny from a meiosis in which a nucleus carrying the new allele took part. The earliest expression of this allele could be during the formation of a new ascus when the allele could be involved in an effect inherited through the cytoplasm to all progeny ascospores. The allele might also be inherited as a typical nuclear locus and segregate into one half of

the ascospores of each relevant ascus and affect the aerialogenesis phenotypes of only one half of the spore pairs. Or it might have two effects, one through the cytoplasm to all progeny ascospores and one as a nuclear locus, further affecting only the strains carrying it in their nuclei. This implies that either strain 59-4 or 8-1 was heterokaryotic for the new and old alleles at this locus, maintaining the old phenotype themselves, but passing on new genetic combinations, and hence possible new gene interactions to their progeny. The fact that two types of progeny came from the same cross tube (same parents) in cross 59-4 x 8-1 indicates that one of the parents was heterokaryotic. The simplest explanation would be one which explains the segregations and loci up to the point of cross 7 Type III asci and then invokes one new allele which will allow for an explanation of the cross 7 Type III asci and as many as possible of the asci from crosses between strains of ascus 240.

The single locus *(aer-4)* associated with maximum aerialogenesis has already been characterized. The two interacting loci present in the original cross and separate from *aer-4* in cross 7 will now be characterized for possible linkage relationships by leaving out the data from Type III asci of cross 7 and all the asci from

than 99% of the progeny, exhibiting this quantitative character, is the closest approximation that could be generated without resorting to models requiring more new alleles. Hypothesizing one new allele arising during a study of this length and magnitude is quite acceptable to me, when considering known somatic and meiotic mutation rates and the previous genetic variation found by Mylyk (1971). Proposing more than one new allele is not an acceptable alternative hypothesis considering the quantitative nature of the character being studied and the variability shown in the measurements. If only two spore pairs out of over 1000 have been mischaracterized due to the variability in expression of the phenotype or through environmental influences on an unusual genetic combination then these two loci could also be explainable. Another possible explanation would be that there are other loci closely linked to those proposed or to each other which are almost always in one combination but in these two asci they have undergone recombination, thus generating a novel and as yet unexplainable combination. This later explanation is also quite possible when one considers the numerous metabolic events, and hence gene loci, which must be involved in the process under consideration.

The frost locus *(fr)* on LG I, which has been

ylo — *aer-1* — *aer-2*	13 Co
aer-2 — *aer-1* — *ylo*	14 Co
ylo — *aer-1* — *aer-2*	15 Co

When comparing these orders with the segregations found among the Type I and II progeny shown in Table 13, which do not exhibit a NG phenotype, the first order is most likely. The Type I progeny, from cross 8-1 x 59-4, contain 2 asci which are first division segregants for *ylo* and second division segregants for aerial and one ascus second *ylo* and first aerial . The Type II progeny, from cross 59-4 x 8-1, have two asci which are first division segregants for *ylo* and second for aerial and one second *ylo* and first aerial. There are no asci observed which are second division segregants for both *ylo* and aerial, suggesting the two linked aerialogenesis loci are on the opposite side of the centromere from *ylo*. From Table 8 (in the Results Chapter), where strain 8-5 is hypothesized to contain both *aer-1* and *aer-2*, there is no evident qualitative effect of these loci on conidiogenesis. The information in Table 9 (in the Results Chapter), indicates a possible quantitative effect of these loci on conidiogenesis. It is proposed that *aer-1* and *aer-2* are present in the first two spore pairs of ascus 59 and these two spore pairs exhibit a greatly increased amount of conidiogenesis in comparison to the

second two spore pairs, proposed to contain *aer-1*$^+$ and *aer-2*$^+$. From the quantitative variation of conidiogenesis in the parental strains, shown in Table 9, I would propose that strain A2 also contains *aer-1* and *aer-2* while OR4 contains the wild type alleles at these loci. These same correlations between the *aer-1* and *aer-2* alleles and conidiogenesis will be discussed further in a later consideration of ascus 240.

The segregation patterns observed in Tables 14 through 17 (in the Results Chapter) indicate that the *ylo* phenotype and the maximum aerialogenesis phenotype are unlinked, with twelve PD and thirteen NPD asci of the 63 asci collected from these crosses. If the progeny from these four crosses had been isolated as random spores, a model could have been justified of one locus with two alleles present in strain 59-4, one allele giving aerial and one the 8-1 relatively nonaerial phenotype. The cross 7, Type III progeny and the progeny of crosses between strains from ascus 240 could then be explained as caused by one new mutation unlinked to *ylo*, which was now interacting with the alleles previously present and linked to *ylo*. Treating the results of crosses 14 through 17 (Tables 14 through 17) as random spores gives 55 strains of the maximum aerialogenesis phenotype, 62 strains 1-3, 63 strains 5-7 and 74 of the

NG phenotype. A chi square test indicates that there is no significant difference at the 0.05 level between these results and 1:1:1:1 segregation with each of four allele combinations (two different alleles at two loci) associated with a different phenotype. However, this points up the value of isolating and analyzing asci. A simple two locus interaction model will not explain a sufficient number of the ascus segregation patterns observed from these crosses and random spore data would not have been able to provide this delineation. With random spores a simple two locus interaction model would have appeared correct and random spore data could not refute it.

Along with the two proposed loci *aer-1* and *aer-2*, closely linked on LG VI, I propose that a mutation in strain 59-4 has made it heterokaryotic for another locus. Mylyk (1971) found a total of eight mutants involved in female sterility, which all appeared to have arisen spontaneously among the strains he was investigating. All of his mutant strains differed from wild type in their superficial aerial growth characteristics and their apparent spontaneous occurrence adds further credence to the proposal of a new allele arising during my studies. This new locus was previously represented by the *aer-3* allele and after the mutational event by either the

aer-3 or *aer-3*$^{+}$ allele. The "+" designation is usually reserved for wild type alleles. However, previous authors (Crocker and Tatum, 1967) have indicated that wild type *Neurospora crassa* does not conidiate or produce aerial hyphae on sorbose medium during a time period comparable to that of these experiments. Hence, in the case of each proposed locus, the "+" allele is the one associated with reduced aerialogenesis. This new allele is associated with a general reduction of growth in several genotypes and specifically supresses aerialogenesis when present with the normally aerial, *aer-1* and *aer-2* alleles. The *aer-3* locus is unlinked to *ylo*, accounting for the newly observed apparent lack of linkage between *ylo* and a maximum aerialogenesis phenotype in later crosses.

Table 18 presents the genotypes I propose, to account for the phenotypic differences observed in my experiments. Genotype to phenotype correlations are presented in Table 19. Genotypic combinations present before the occurrence of a mutation at the *aer-3* locus are given first. Those gene interactions involving *aer-3* and *aer-3*$^{+}$ in later crosses (cross 7, Type III and crosses 14 through 17) are presented below. The *aer-4* locus was segregating two alleles in the earlier crosses but strains 59-4 and 8-1 were selected specifically for a lack of

TABLE 18. Proposed genotypes for strains used as parents in crosses during this study.

A2	*ylo* ⊗ *aer-1* *aer-2*	*aer-3*----*aer-4*$^+$
OR4	*ylo*$^+$ ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*
8-1	*ylo*$^+$ ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*$^+$
8-3	*ylo*$^+$ ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*$^+$
8-5	*ylo* ⊗ *aer-1* *aer-2*	*aer-3*----*aer-4*
8-7	*ylo* ⊗ *aer-1* *aer-2*	*aer-3*----*aer-4*
59-1	*ylo* ⊗ *aer-1* *aer-2*	*aer-3*----*aer-4*
59-4	*ylo* ⊗ *aer-1* *aer-2*	*aer-3/aer-3*$^+$----*aer-4*$^+$
59-5	*ylo*$^+$ ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*$^+$
59-8	*ylo*$^+$ ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*
275-3	*ylo* ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*$^+$
240-1	*ylo*$^+$ ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*$^+$
240-3	*ylo*$^+$ ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*$^+$
240-5	*ylo* ⊗ *aer-1* *aer-2*	*aer-3*$^+$----*aer-4*$^+$
240-7	*ylo* ⊗ *aer-1* *aer-2*	*aer-3*$^+$----*aer-4*$^+$
131-7	*ylo* ⊗ *aer-1*$^+$ *aer-2*$^+$	*aer-3*----*aer-4*

TABLE 19. Proposed aerialogenesis genotypes and their related phenotypes.

aer-1	*aer-2*	*aer-3*	*aer-4*	phenotype	
-	-	-	-	aerial	
-	-	-	+	aerial	
-	+	-	-	aerial	relatively rare, due to SCO between *aer-1* and *aer-2*
-	+	-	+	1-3	
+	-	-	-	aerial	
+	-	-	+	1-3	
+	+	-	-	aerial	
+	+	-	+	1-3	

Phenotypes when *aer-3*$^+$ is present in one of the parental nuclei giving rise to the ascus (*aer-4*$^+$ is always present).

-	-	-	+	aerial	
-	-	+	+	5-7	
-	+	-	+	5-7	relatively rare, due to SCO between *aer-1* and *aer-2*
-	+	+	+	NG	
+	-	-	+	NG	
+	-	+	+	NG	
+	+	-	+	1-3	
+	+	+	+	NG	

SCO - single cross-over

the *aer-4* allele, hence only the *aer-4*$^{+}$ allele is present in crosses 7, 14, 15, 16, and 17.

Strains 59-4, 240-5 and 240-7 all have the same alleles present on LG VI for aerialogenesis loci in this model. These strains also have very similar amounts of conidiogenesis as illustrated in Tables 9 and 10. In asci 59 and 240 there appears to be a correlated segregation of *aer-1* and *aer-2* with conidia production. This correlation is observed even though the strains containing *aer-1* and *aer-2* in ascus 240 have their aerialogenesis greatly reduced due to mutation at a third locus. I would suggest that either *aer-1*, *aer-2*, or another allele on LG VI has a significant affect on conidiogenesis, separate from any phenotypic affects on aerialogenesis. The fact that strains 59-7 and 59-8 (*aer-1*$^{+}$, *aer-2*$^{+}$) were also greatly reduced in conidiogenesis, even though they each were of the maximum aerialogenesis phenotype on sorbose, would also suggest that the two events are controlled by different genetic elements.

The proposed genotype:phenotype relationships will account for all but two of the asci observed in this study. One of the unexplained asci is number 642 from cross 16 and the other is 622 from cross 17. Bearing in mind that 265 asci were analyzed in this study out of the over 650 isolated, a model which can account for greater

than 99% of the progeny, exhibiting this quantitative character, is the closest approximation that could be generated without resorting to models requiring more new alleles. Hypothesizing one new allele arising during a study of this length and magnitude is quite acceptable to me, when considering known somatic and meiotic mutation rates and the previous genetic variation found by Mylyk (1971). Proposing more than one new allele is not an acceptable alternative hypothesis considering the quantitative nature of the character being studied and the variability shown in the measurements. If only two spore pairs out of over 1000 have been mischaracterized due to the variability in expression of the phenotype or through environmental influences on an unusual genetic combination then these two loci could also be explainable. Another possible explanation would be that there are other loci closely linked to those proposed or to each other which are almost always in one combination but in these two asci they have undergone recombination, thus generating a novel and as yet unexplainable combination. This later explanation is also quite possible when one considers the numerous metabolic events, and hence gene loci, which must be involved in the process under consideration.

The frost locus *(fr)* on LG I, which has been

implicated in alterations of glucose-6-phosphate dehydrogenase (G-6-PD) activity; has some unusual morphological features. On glucose minimal medium, the *fr* strain grows faster than *col-2* or *bd*, the other two loci involved in controlling G-6-PD activity, and forms aerial hyphae which later collapse and branch extensively along the medium (Brody, 1973). This pattern is altered when the frost strain is grown upside down, with the medium on top, as was the case in most of my experiments. With incubation upside down the *fr* strain has less aerial growth and a much slower growth rate than before. In my experiments the colonies were almost always inoculated and incubated with the medium on top (i.e. upside down) for ease of handling. In the few cases where inoculation and/or incubation with the medium on the bottom were tried, aerialogenesis of the aerial strains was followed by collapse of the aerials and growth along the medium until more aerials were formed. This growth pattern would certainly allow for greater increase in colony diameter than I observed in my upside down colonies but would have also made it impossible to measure aerial extension growth as I have done.

Noting changes in chemical components and gene loci and correlations with gross or ultrastructural alterations of phenotype provide a direction in which to

search, but have certainly not provided the answers to major questions. Realizing the canalization and compartmentation present in *Neurospora*, perhaps one of the more interesting questions would be: given that a single locus or several loci interacting can have distinct affects on morphology, what effect does that altered phenotype have on other biochemical or even genetic regulatory events within the organism? In this study several loci were seen to contribute to aerial hyphae production, with different genotypes producing quantitatively different amounts of morphological alterations in the phenotype. Perhaps a series of mutants, similar to the ones found in this study, could be employed in defining how much morphological change can affect various physiological processes. With the quantification of morphological effect demonstrated here a correlated response of physiological parameters to morphological change might be more discernable.

APPENDIX A

The computer output, referred to in this section, will be found in the pocket inside the back cover of this volume.

This section contains computer output illustrative of both the program used and the output received in analyzing the data of this study. The example employs asci 472 and 473 from cross 240-1 x 240-5 (cross 14, Tables 11 and 14 in the Results Chapter. Ascus 472 is a parental 2:2 ascus for aerial growth type with first division segregation for *ylo/ylo*$^+$ (spore pairs one and two *ylo*). First division segregation was also observed for *ylo/ylo*$^+$ in ascus 473, with spore pairs three and four *ylo*, while a typical TT segregation pattern was present for the aerial growth types.

The printout first lists the computer program as described in the Materials and Methods, Section III Computer Analysis. This is followed by messages generated by the compiler and loader. The program output comes next, giving the data from the two asci and their parents in this example in the various configurations generated by the analysis, as described

previously. First the GCN values for single strains are shown in a section headed "ASCI" with their accompanying standard deviations (SD3) and standard errors (SE1). Next, the plots of the strains of the first ascus (472 in this example) plus the parents, and the second ascus (473) plus parents are shown. In all the plots the Y axis is time, with the bottom line of symbols being the 48 hr reading, the next line up, the 54 hr reading, etc.; up to the top line, which represents the 96 hr reading. The X axis, on all plots, extends from a GCN value of 0.000 to 4.500. The theoretical maximum GCN is 4.000, a maximum aerial growth observation of 4 for all inocula of a given strain at a certain time, but allowing the X axis to continue to 4.500 creates an easier frame for reading the plots. In all the plots the parents are represented by the numbers "1" and "2". In these particular plots strain 240-1 is represented by the plotted "2's" and strain 240-5 by the plotted "1's". In the first plot the 8 spores of the first ascus (472) are represented by the plots of the first 8 letters of the alphabet, A through H, respectively. The second plot contains the second ascus (473), with its 8 spores represented by the letters J through Q,

respectively. Hence, in the first plot, for instance, E and F would represent the two members of the third spore pair in the first ascus (strains 472-5 and 6), while in the second plot N and O would represent the two members of the third spore pair in the second ascus (strains 473-5 and 6). A not equal sign (≠) in any of the plots indicates that more than one of the strains has a coincident point in that position.

The third and fourth plots contain the same parental plots, with the same symbols ("1" and "2"), as well as plots of the combined data for each spore pair. In the third plot the two parents (parent 240-5 represented by "1" and 240-1 by "2") are shown along with the four spore pairs of the first ascus (472). The four spore pairs are represented by the first four letters of the alphabet, A through D, respectively. The fourth plot contains the two parents ("1" and "2"), plus the four spore pairs of the second ascus (473), represented by the letters J through M, respectively. In plots three and four, the spore pair and parent symbols have been connected by lines, for greater ease of observation. The parental strain, 240-5, is shown by a black dashed line while the 240-1 parent is indicated with a black solid line. The four spore pairs

in each plot are shown as solid, red, blue, green and orange lines, respectively. The GCN values for the lumped spore pair data are found next, to complete the GCN analysis.

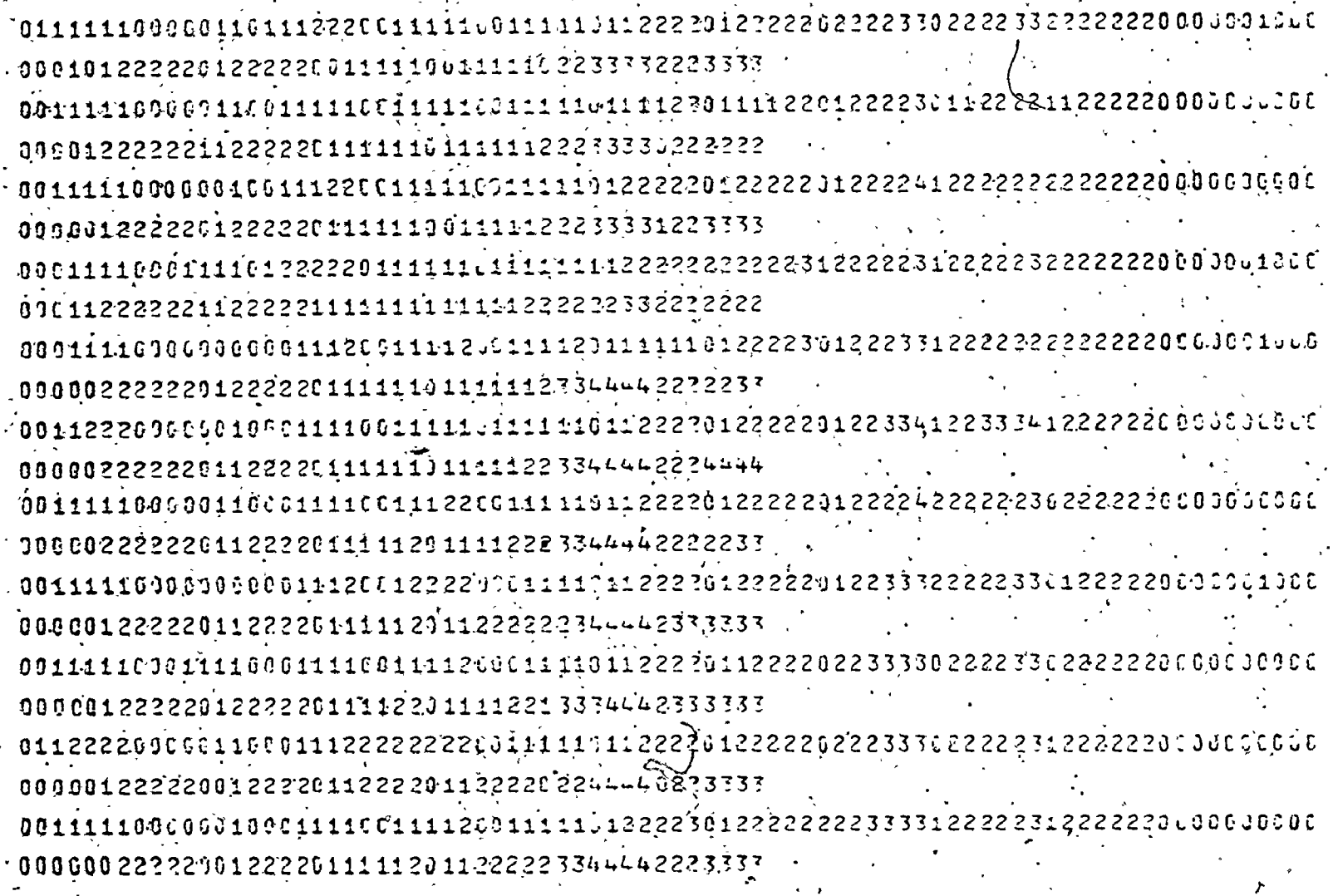
The TGN analysis then begins with a printout of I, NUM, MEAN (TGN value), SD and SE. I is the number indexing the strains found on single plate replicates, with I = 1 being the first parent (240-5, in this example) and I = 10, the second parent (240-1) on the plate. I = 2,...,9 indicates the eight spore pairs of the first ascus, respectively (472), while I = 11,...18 indexes the eight spore pairs of the second ascus (473). NUM counts the number of replicate platings of each strain in the experiment. The MEAN is the TGN value for the strain, found by adding together all the growth readings for the strain in question and dividing by NUM. SD and SE are the standard deviation and standard error values for each of the MEAN (TGN) calculations.

The unsorted data are then shown as inoculum TGN values for each plating of each strain as a means of verifying calculations. The raw numerical data, originally input for these exemplary asci to facilitate checking of these calculations, are given in Table 20. The data are arranged by petri-plate replicate with 20

Table 20. Observational data grouped by petri-plate replicate for the example presented in the appendix (asci 472 and 473 and the parents with each group of seven numbers being the seven observations for a different strain).


```
000111101122220122222001111109111111012222201222220222223012222222222220000010000
000001222220122222011111201111122233333222344
001111101122220111122000111110001111101222220122222011222201112221222222000001000
000001222220112222011111201111121222233222223
000111100111110011111100111110001111101222220112222022222201112221111122000000000
000001112220111222011111110111111122222212222222
000111101112220112222000111100011111011222201222220122223011222212222220000010000
000101222220122222011111101111112222333222222
001112200111120011122001111200111110122222012222201223330122222222222200000000000
000001222220112222011222201222222233330222333
000111100111110012222000111130011110012222002222200223330022333002222200000000000
000000222220022222001111200111222333444222223
001111100111110011111000111100111110122222011222201222240112222222222200000010000
000101112222011122200111110111111222334412222333
000222200000011012222201111120000111022222202233331223444022233422222200000000000
000101222220122222011122201222222334444233344
001222000000110012222011222200111220222222012222220222333022223332222220000000000
000201222220122222011122201122222234444222444
```

Table 20. (continued).



replicates in this example. Within each petri-plate replicate are 126 readings taken from that plate, representing the seven time readings for each of 18 strains. The first seven are from parent one, the first being the 48 hr reading, the second 54 hr and so on, to the seventh number the 96 hr reading. The next eight groups of seven numbers are the strains of the first ascus, 472 in this case, followed by the second parent and the eight strains of the second ascus. See also a further explanation of data input in Materials and Methods, Section III Computer Analysis).

Following the unsorted data in the output are the Student's t-test calculations based on TCN values for strains, parents, and combined spore pair data: M indexes the spore pairs, with an M value from 1 to 4 being the four spore pairs of the first ascus (472), and an M value from 5 to 8 being the 4 spore pairs of the second ascus. (For a further explanation of the relationship between the indices J,K,M and I, refer to Materials and Methods, Section III, Part B, and Figure 2). All abbreviations beginning in STUDT represent the values of Student's t-tests with the letter or number following, indicating which groups of data are being compared. STUDT1 compares the two

members of the spore pair. STUDTP and STUDTQ compare the first member of the spore pair with the first and second parents, respectively, while STUDT2 and STUDT3 compare the second member of the spore pair with the two parent strains, respectively. NUM2 counts the total number of inocula for the strains of a spore pair and AMEAN is the TGN value of the spore pair. AMEAN is calculated by summing all the growth readings for both members of a spore pair (TGN4) and dividing by NUM2. TGN4 is the sum of the observations of all the growth readings for a strain and TGN5 is the sum of the squares of all these observations. SD2 is the standard deviation value for the AMEAN of the strain. STUDT4 and STUDT5 compare the combined spore-pair data with the data of the first and second parents, respectively, using Student's t-tests.

REFERENCES

ABRAMSKY, T., W. A. SCOTT, E. L. TATUM. 1971. Neurospora Morphology and 6-Phosphogluconic Acid Dehydrogenase. Fed. Proc. 30: 1069 abstr.

BARRATT, R. W. and L. GARNJOBST. 1949. Genetics of a Colonial Microconidiating Mutant Strain of *Neurospora crassa*. Genetics 34: 351-369.

BARRATT, R. W., W. N. OGATA. 1970. Neurospora Stock List. Neurospora Newsl. 16: 23-123. 5th revision.

BARRATT, R. W., W. N. OGATA. 1975. Neurospora Stock List. Neurospora Newsl. 21: 33-91. 7th revision.

BARRATT, R. W. and A. RADFORD. 1969. Genetic Markers, Linkage Groups, and Enzymes in *Neurospora crassa*. Neurospora Newsl. 14: 27-38.

BARRATT, R. W. and A. RADFORD. 1970. Genetic Markers, Linkage Groups, and Enzymes in *Neurospora crassa* - Supplement 1. Neurospora Newsl. 16: 19-22.

BARTNICKI-GARCIA, S. 1973. "Fundamental Aspects of Hyphal Morphogenesis." In Microbial Differentiation. Cambridge Univ. Press (London, England and New York, New York, U.S.A.) x +450 pp.

BAYLEY, M. Editor. 1975 (Prerelease). McMaster University Computing Center User's Guide.

BERLINER, M. D., P. W. NEURATH. 1965. The Band Forming Rhythm of *Neurospora* Mutants. J. Cell. Comp. Physiol. 65: 183-194.

BERLINER, M. D., P. W. NEURATH. 1966. Control of Rhythmic Growth of *Neurospora* "Clock" Mutant by Sugars. Can. J. Microbiol. 12: 1068-1070.

BIANCHI, D. E. 1964. An Endogenous Circadian Rhythm in *Neurospora crassa*. J. Gen. Microbiol. 35: 437-445.

BISHOP, O. N. 1971. Statistics for Biology. Second Edition. Longman Group Ltd. (London) viii + 216 pp.

BROCKMAN, H. E., F. J. DESERRES. 1963. "Sorbose Toxicity" in *Neurospora*. Am. J. Bot. 50: 709-714.

BRODY, S. 1970. Correlation Between Reduced Nicotinamide Adenine Dinucleotide Phosphate Levels and Morphological Changes in *Neurospora crassa*. J. Bacteriol. 101: 802-807.

BRODY, S. 1973. Metabolism, Cell Walls, and Morphogenesis. In S. Coward (Ed.) Developmental Regulation. Aspects of Cell Differentiation. Acad. Pr., N.Y.

BRODY, S., S. HARRIS. 1973. Circadian Rhythms in *Neurospora*: Spatial Differences in Pyridine Nucleotide Levels. Science 180: 498-500.

BRODY, S. and E. L. TATUM. 1966. The Primary Biochemical Effect of a Morphological Mutation in *Neurospora crassa*. Proc. Nat. Acad. Sci. U.S.A. 56: 1290-1297.

BRODY, S. and E. L. TATUM. 1967. Phosphoglucomutase Mutants and Morphological Changes in *Neurospora crassa*. Proc. Nat. Acad. Sci. U.S.A. 58: 923-930.

CROCKEN, B. and E. L. TATUM. 1967. Sorbose Transport in *Neurospora crassa*. Biochim. Biophys. Acta. 135: 100-105.

CROCKEN, B. and E. L. TATUM. 1968. The Effect of Sorbose on Metabolism and Morphology of *Neurospora*. Biochim. Biophys. Acta. 156: 1-8.

DELMER, D. P., S. BRODY. 1975. Circadian Rhythms in *Neurospora crassa*: oscillation in the level of adenine nucleotide. J. Bacteriol. 121: 548-553.

DE TERRA, N. and E. L. TATUM. 1961. Sorbose and Enzyme Alter the Composition of the Cell Wall and Induce Morphological Changes. Science 134: 1066-1068.

DE TERRA, N. and E. L. TATUM. 1963. A Relationship Between Cell Wall Structure and Colonial Growth in *Neurospora crassa*. Am. J. Bot. 50: 669-677.

DICKER, J. W., N. OULEVEY, G. TURIAN. 1969. Amino Acid Induction of Conidiation and Morphological Alterations in Wild Type and Morphological Mutants of *Neurospora crassa*. Arch. Mikrobiol. 65: 241-257.

DICKER, J. W., R. PEDUZZI, G. TURIAN. 1975. Studies on the Conidial Differentiation of *Neurospora crassa* - VI: Functional Anomalies of the Conditional Aconidial Mutant "Amycelial". Ann. Microbiol. A126: 409-420.

FELDMAN, J. F., M. N. HOYLE. 1973. Isolation of Circadian Clock Mutants of *Neurospora crassa*. Genetics 75: 605-613.

FINCHAM, J. R. S., P. R. DAY. 1971. Fungal Genetics, 3rd ed., Blackwell Sci. Pub. (Oxford), 402 p.

GARNJOBST, L., E. L. TATUM. 1967. A Survey of New Morphological Mutants in *Neurospora crassa*. Genetics 57: 579-604.

GRIGG, G. W. 1958. The Genetic Control of Conidiation in a Heterokaryon of *Neurospora crassa*. J. Gen. Microbiol. 19: 15-22.

GRIGG, G. W. 1960a. The Control of Conidial Differentiation in *Neurospora crassa*. J. Gen. Microbiol. 22: 662-666.

GRIGG, G. W. 1960b. Temperature-Sensitive Genes

Affecting Conidiation in *Neurospora*. J. Gen. Microbiol. 22: 667-670.

HALABAN, R. 1975. Glucose Transport-Deficient Mutant of *Neurospora crassa* With an Unusual Rhythmic Growth Pattern. J. Bacteriol. 121: 1056-1063.

HOCHBERG, M. L., M. L. SARGENT. 1974. Rhythms of Enzyme Activity Associated with Circadian Conidiation in *Neurospora crassa*. J. Bacteriol. 120: 1164-1175.

KLINGMULLER, W., H. HUH, F. KAUDEWITZ. 1970. Regulierende Wirkung Des Gens C Der Sorbose-Resistenz von *Neurospora crassa* Auf Den Zuckertransport. (Regulatory Function of Sorbose Resistance Gene C in Sugar Transport of *Neurospora*). Mol. Gen. Genet. 109: 27-41.

KLINGMULLER, W., F. KAUDEWITZ. 1967. "No Growth" in Forced Heterokaryons From Sorbose Resistant (Transport Defective) *Neurospora crassa* Mutants. Z. Naturforsh 226: 1024-1027.

KOCH, A. L. 1975. Kinetics of Mycelial Growth. J. Gen. Microbiol. 89: 209-216.

LEHNINGER, A. L. 1970. Biochemistry. Worth Publishers Inc. (New York) XIII + 833 pp.

MAHADEVAN, P. R., U. R. MAHADKAR. 1970. Role of Enzymes in Growth and Morphology of *Neurospora crassa*:

Cell Wall Bound Enzymes and Their Possible Role in Branching. J. Bacteriol. 101: 941-.

MAHADEVAN, P. R. and E. L. TATUM. 1965. Relationship of the Major Constituents of the *Neurospora crassa* Cell Wall to Wild Type and Colonial Morphology. J. Bacteriol. 90: 1073-1081.

MAHADEVAN, P. R. and E. L. TATUM. 1967. Localization of Structural Polymers in the Cell Wall of *Neurospora crassa*. J. Cell Biol. 35: 295-302.

MARTENS, C. L., M. L. SARGENT. 1974. Circadian Rhythms of Nucleil Acid Metabolism in *Neurospora crassa*. J. Bacteriol. 117: 1210-1215.

MATSUYAMA, S. S., R. E. NELSON, R. W. SIEGEL. 1974. Mutations Specifically Blocking Differentiation of Macroconidia in *Neurospora crassa*. Develop. Biol. 41: 278-287.

MAURER, H. A., M. R. WILLIAMS. 1972. A Collection of Programming Problems and Techniques. Prentice-Hall Inc. (Englewood Cliffs, New Jersey) X + 256 pp.

McCRACKEN, D. D. 1972. A Guide to Fortran IV Programming. John Wiley and Sons Inc. (New York) XIII + 289 pp.

MISHRA, N. C. 1976. Genetics and Biochemistry of Morphogenesis in *Neurospora*. Accepted for publication in Adv. Genet. 19.

MISHRA, N. C. and E. L. TATUM. 1970. Phosphoglucomutase Mutants of *Neurospora sitophila* and Their Relation to Morphology. Proc. Nat. Acad. Sci. U.S.A. 66: 638-645.

MISHRA, N. C. and E. L. TATUM. 1972. Effect of L-Sorbose on Polysaccharide Synthesis of *Neurospora crassa*. Proc. Nat. Acad. Sci. U.S.A. 69: 313-317.

MURAYAMA, T., T. ISHIKAWA. 1973. Mutation in *Neurospora crassa* Affecting Some of the Extracellular Enzymes and Several Growth Characteristics. J. Bact. 115: 796-804.

MURAYAMA, T., T. ISHIKAWA. 1975. Characterization of *Neurospora crassa* Mutants Deficient in Glucose-phosphate Isomerase. J. Bacteriol. 122: 54-58.

MURRAY, J. C. and A. M. SRB. 1962. The Morphology and Genetics of Wild-Type and Seven Morphological Mutant Strains of *Neurospora crassa*. Can. J. Bot. 40: 337-350.

MYLYK, O. 1971. A Genetic Study of Female Sterility in *Neurospora crassa*. Ph.D. Thesis, McMaster University (Hamilton, Canada).

NELSON, R. E., C. P. SELITRENNIKOFF, R. W. SIEGEL. 1975a. Mutants of *Neurospora* Deficient in Nicotinamide Adenine Dinucleotide Phosphate Glycohydrolase. J. Bacteriol. 122: 695-709.

NELSON, R. E., C. P. SELITRENNIKOFF, R. W. SIEGEL. 1975b. Cell changes in *Neurospora*. In "Cell Cycle and Cell Differentiation" (J. Reinert and H. Holzer, eds.). Vol. 7, pp290-310. Springer-Verlag, Berlin.

NELSON, R. E., C. P. SELITRENNIKOFF, R. W. SIEGEL. 1976. Developmental Regulation of Nicotinamide Adenine Dinucleotide (Phosphate) Glycohydrolase in *Neurospora crassa*. Dev. Biol. 50: 122-133.

PINA, E., E. L. TATUM. 1967. Inositol Biosynthesis in *Neurospora crassa*. Biochim. Biophys. Acta. 136: 265-271.

RAND, J. B. 1975. The Regulation of Sugar Transport and Galactose Metabolism in *Neurospora crassa*. Ph.D. Thesis, The Rockefeller Univ. (N.Y.).

RIZVI, S. R. H. and N. F. ROBERTSON. 1965. Apical Disintegration of Hyphae of *Neurospora crassa* as a Response to L-Sorbose. Trans. Brit. Mycol. Soc. 48: 469-477.

ROBERTSON, N. F. and S. R. H. RIZVI. 1968. Some Observations on the Water-Relations of the Hyphae of *Neurospora crassa*. Ann. Bot. 32: 279-291 (1968).

ROHLF, F. J., R. S. SOKAL. 1969. Statistical Tables. W. H. Freeman and Company (San Francisco) XI + 253 pp.

ROSSIER, C., N. OULEVEY, G. TURIAN. 1973. Electron Microscopy of Selectively Stimulated

Microconidiogenesis in Wild Type *Neurospora crassa*. Arch. Mikrobiol. 91: 345-354.

RUSSELL, P. J., A. M. SRB. 1974. A Study of L-Glutamine: D Fructose 6-Phosphate Amidotransferase in Certain Developmental Mutants of *Neurospora crassa*. Mol. Gen. Genet. 129: 77-86.

SARGENT, M. L., W. R. BRIGGS, D. O. WOODWARD. 1966. The Circadian Nature of a Rhythm Expressed by an Invertaseless Strain of *Neurospora crassa*. Plant Physiol. 41: 1343-1349.

SARGENT, M. L., D. O. WOODWARD. 1969. Genetic Determinants of Circadian Rhythmicity in *Neurospora*. J. Bacteriol. 97: 861-866.

SCHEFLER, W. C. 1969. Statistics for the Biological Sciences. Addison-Wesley Publishing Company (Don Mills, Ontario) 231 pp.

SCHMIT, J. C., S. BRODY. 1975. Developmental Control of Glucosamine and Galactosamine Levels During Conidiation in *Neurospora crassa*. J. Bacteriol. 122: 1071-1075.

SCOTT, W. A., T. ABRAMSKY. 1973. *Neurospora* 6-Phosphogluconate Dehydrogenase II. Properties of two Purified Mutant Enzymes. J. Biol. Chem. 248: 3535-3545.

SCOTT, W. A. and E. L. TATUM. 1970. Glucose-6-Phosphate Dehydrogenase and *Neurospora* Morphology. Proc.

Nat. Acad. Sci. U.S.A. 60: 515-522.

SELITRENNIKOFF, C. P., R. E. NELSON, R. W. SIEGEL. 1974. Phase-Specific Genes for Macroconidiation in *Neurospora crassa*. Genetics 78: 679-690.

SIEGEL, R. W., S. S. MATSUYAMA, J. C. UREY. 1968. Induced Macroconidial formation in *Neurospora crassa*. Experientia 24:1179-1181.

SOKAL, R. R., F. J. ROHLF. 1969. Biometry. W. H. Freeman and Company (San Francisco) XXI +776 pp.

TATUM, E. L., R. W. BARRATT and V. M. CUTTER. 1949. Chemical Induction of Colonial Paramorphs in *Neurospora* and *Syncephalastrum*. Science 109: 509-511.

TERENZI, H. F., M. M. FLAWIA, T. TELLEZ-INON, H. N. TORRES. 1976. Control of *Neurospora crassa* Morphology by Cyclic Adenosine 3'5'-Monophosphate and Dibutyryl Cyclic Adenosine 3'5'-Monophosphate. J. Bacteriol. 126: 91-99.

TERENZI, H. F., M. M. FLAWIA , H. N. TORRES. 1974. A *Neurospora crassa* Morphological Mutant Showing Reduced Adenylate Cyclase Activity. Biochem. Biophys. Res. Commun. 58: 990-996.

TRINCI, A. P. J. 1974. A Study of the Kinetics of Hyphal Extension and Branch Initiation of Fungal Mycelia. J. Gen. Microbiol. 81: 225-236.

TRINCI, A. P. J., A. COLLINGE. 1973a. Influence of L-Sorbose on the Growth and Morphology of *Neurospora crassa*. J. Gen. Microbiol. 78: 179-193.

TRINCI, A. P. J., A. J. COLLINGE. 1973b. Structure and Plugging of Septa of Wild Type and Spreading Colonial Mutants of *Neurospora crassa*. Arch. Mikrobiol. 91: 355-364.

TRINCI, A. P. J., A. J. COLLINGE. 1975. Hyphal Wall Growth in *Neurospora crassa* and *Geotrichum candidum*. J. Gen. Microbiol. 91: 355-361.

TURIAN, G. 1966. "Morphogenesis in Ascomycetes" In G. C. Ainsworth and A. S. Sussman (Eds.) The Fungi: An Advanced Treatise. Vol 2. Acad. Pr. (New York, New York).

TURIAN, G. and N. MATIKIAN. 1966. Conidiation of *Neurospora crassa*. Nature (London) 212: 1067-1068.

TURIAN, G., N. OULEVEY, M. CORTAT. 1973. Studies on the Conidial Differentiation of *N. crassa*. V. Ultrastructure of the Macroconidiogenesis Sequence. Ann. Microbiol. 124: 444-458.

UREY, J. C. 1971. Enzyme Patterns and Protein Synthesis During Synchronous Conidiation in *Neurospora crassa*. Dev. Biol. 26: 17-27.

VELDMAN, D. J. 1967. Fortran Programming for the Behavioral Sciences. Holt, Rinehart and Winston (New York, U.S.A.). X +406 pp.

VOGEL, H. J. 1956. A Convenient Growth Medium for *Neurospora* (Medium N). Microb. Genet. Bull. 13: 42-43.

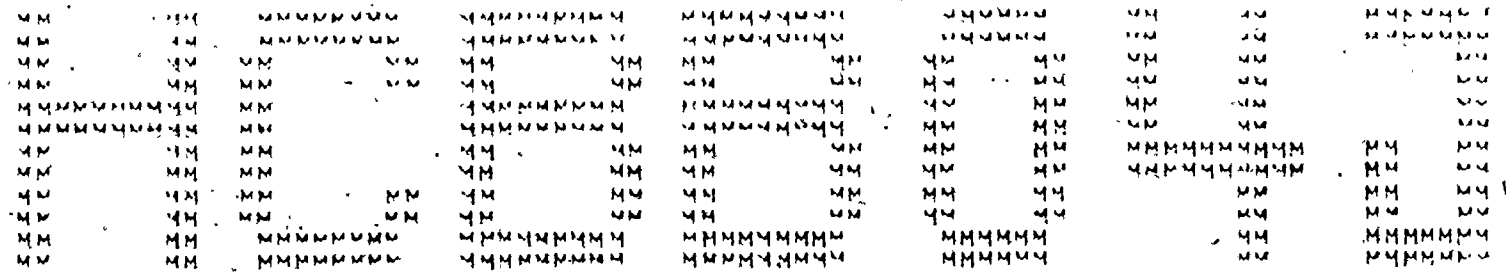
WEISS, B. and G. TURIAN. 1966. A Study of Conidiation in *Neurospora crassa*. J. Gen. Microbiol. 44: 407-418.

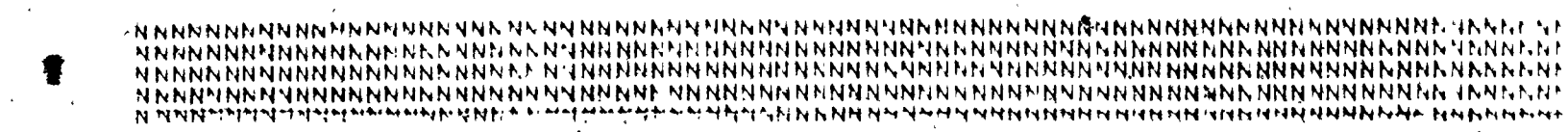
WEST, D. J. 1975. Effects of pH and Biotin on a Circadian Rhythm of Conidiation in *Neurospora crassa*. J. Bacteriol. 123: 387-389.

WESTERGAARD, M. and H. K. MITCHELL. 1947. *Neurospora* V. A Synthetic Medium Favoring Sexual Reproduction. Am. J. Bot. 34: 573-577.

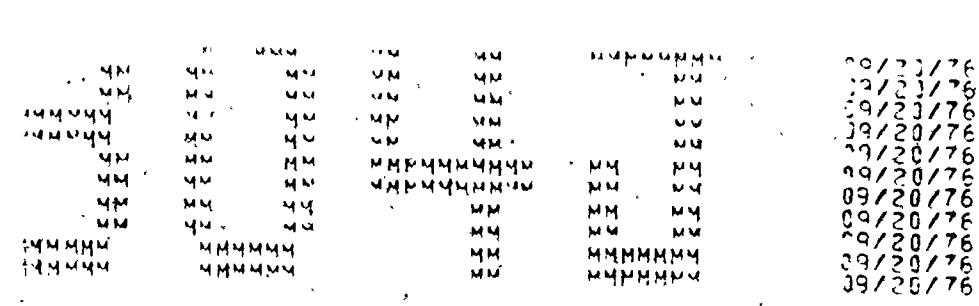
WRATHALL, C. R., E. L. TATUM. 1973. The Peptides of the Hyphal Wall of *Neurospora crassa*. J. Gen. Microbiol. 78: 139-155.

WRATHALL, C. R., E. L. TATUM. 1974. Hyphal Wall Peptides and Colonial Morphology in *Neurospora crassa*. Biochem. Genet. 12: 59-68.






```
      PROGRAM TST (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
C
C DEFINE ALL THE ARRAYS
      DIMENSION IDATA(18,7,20),GCN(18,7),NUM(18),TGN1(18,20)
      DIMENSION TGN2(18),TGN3(18),AMEAN(18),SD(18),SE(18)
        DIMENSION STUDT1(8), STUDTP(8), STUDTQ(8), STUDT2(8),
        DIMENSION NUM2(8),AMEAN2(8),TGN4(8), TGN5(8), SD2(8)
      DIMENSION GCN1(18,7), GCN2(18,7), SD3(18,7), SE1(18,7), SI
        DIMENSION STUDT5(8)
      DIMENSION GCNS(8,7)
C
C SET ALL VALUES IN ARRAY GCNS AT 0.0
      DO 49 M=1,8
        DO 49 J=1,7
          GCNS(M,J)=0.0
49    CONTINUE
C
C SET ALL VALUES IN ARRAYS AT 0.0
999   DO 51 I=1,18
        DO 51 J=1,7
        GCN1(I,J)=0.0
        GCN2(I,J)=0.0
        SD3(I,J)=0.0
        SE1(I,J)=0.0
          GCN(I,J)=0.0
51    CONTINUE
C
C SET ALL VALUES IN ARRAYS AT 0.0
        DO 55 M=1,8
        STUDT4(M)=0.0
        STUDT5(M)=0.0
        STUDT1(M)=0.0
        STUDTP(M)=0.0
        STUDTQ(M)=0.0
        STUDT2(M)=0.0
        STUDT3(M)=0.0
        NUM2(M)=0
        AMEAN2(M)=0.0
        TGN4(M)=0.0
        TGN5(M)=0.0
        SD2(M)=0.0
55    CONTINUE
C
C SET ALL VALUES IN ARRAYS AT 0.0
      DO 61 I=1,18
        NUM(I)=0
        TGN2(I)=0.0
        TI          C
```



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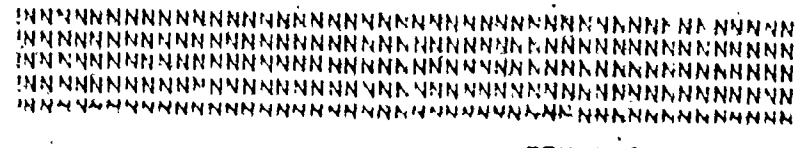

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```
UT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)

S
18,7,20),GCN(18,7),NUM(18),TGN1(18,20)
8),TGN3(18),AMEAN(18),SD(18),SE(18)
*1(8), STUDTP(8), STUDTQ(8), STUDT2(8), STUDT3(8)
(8),AMEAN2(8),TGN4(8), TGN5(8), SD2(8)
8,7), GCN2(18,7), SD*(18,7),SE1(18,7), STUDT4(8)
T5(8)
,7)

SAY GCNS AT 0.

0

RAYS AT 0.0

RAYS AT 0.0

AYS AT 0.0
```

```
      AMEAN2(M)=0.
      TGN4(M)=0.0
      TGN5(M)=0.0
      SD2(M)=0.0
55    CONTINUE
C
C SET ALL VALUES IN ARRAYS AT 0.0
      DO 61 I=1,18
        NUM(I)=0
        TGN3(I)=0.
        TGN4(I)=0.
        AMEAN(I)=0.
        SD(I)=0.0
        SE(I)=0.0
```

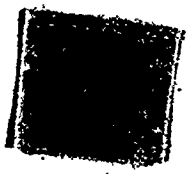
34

PROGRAM TST 77/77 OS FTN 4.6+42

```
61    CONTINUE
C
C SET ALL VALUES IN ARRAYS AT 0.0
      DO 71 I=1,18
        DO 71 K=1,20
          TGN1(I,K)=0.0
71    CONTINUE
C
C TITLE FIRST PART OF PRINTOUT
      WRITE(6,19)
19    FORMAT(1H1,4HASCI,//)
      IG=0
C ENTER DATA
      DO 1 K=1,20
        READ(5,100)((IDATA(I,J,K),J=1,7),I=1,18)
1
100   FORMAT(80I1)
C
C
      DO 11 J=1,7
        DO 11 I=1,18
          DO 15 K=1,20
12
C DEPENDING ON IDATA VALUE,GO TO LABELLED INSTRUCTION
          IF(IDATA(I,J,K)-9)14,15,14
14        IF(J.GE.2) GO TO 16
C COUNT NUMBER OF OBSERVATIONS
          NUM(I)=NUM(I)+1
16        GCN(I,J)=GCN(I,J)+FLOAT(IDATA(I,J,K))
          GCN1(I,J)=GCN(I,J)
          GCN2(I,J)=(GCN2(I,J)+(FLOAT(IDATA(I,J,K))**2))
C GCN2(I,J) CONTAINS SUM OF SQUARED OBSERVATIONS
15        CONTINUE
11    CONTINUE
C GCN CONTAINS TOTALS OF NUMBERS FOR EACH STRAIN AT EACH TIME
C
      DO 21 I=1,18
        DO 21 J=1,7
          IF(NUM(I).EQ.0) GO TO 20
          GCN(I,J)=GCN(I,J)/FLOAT(NUM(I))
C AVERAGE FOR GROWTH CURVE NUMBER
          SD3(I,J)=SQRT(((FLOAT(NUM(I)))*(GCN2(I,J)))-((GCN1(I,
     F(GCN1(I,J)))/((FLOAT(NUM(I)))*((FLOAT(NUM(I)))-1.0)))
C SD3(I,J) CONTAINS STANDARD DEVIATIONS FOR GCN VALUES
          SE1(I,J)=SD3(I,J)/(SQRT(FLOAT(NUM(I))))
C SE1(I,J) CONTAINS STANDARD ERRORS FOR GCN VALUES
          GO TO 21
20          SD3(I,J)=0.0
            SE1(I,J)=0.0
21        CONTINUE
C GCN NOW CONTAINS GROWTH CURVE NUMBERS AS AVERAGES OF TOTAL F
C STRAIN-TIME DIVIDED BY NUMBER OF REPLICATES
C
C PRINTOUT NUM, GCN, J, SD, SE
```

```
RAYS AT 0.0

CTN -.6+42.   09/19/7_ 14.05.40   PAGE   2

RAYS AT J.

PRINTOUT
SCT,//).

DATA(I,J,K),J=1,7),I=1,18)

 20
ALUE,GO TO LABELLED INSTRUCTION
J,K)-9)14,15,14
GO TO 16
RVATIONS
)+1
(I,J)+FLOAT(IDATA(I,J,K))
I,J)
2(I,J)+(FLOAT(IDATA(I,J,K))**2))
SUM OF SQUARED OBSERVATIONS

OF NUMBERS FOR EACH STRAIN AT EACH TIME

0.0)  GO TO 2
(I,J)/FLOAT(NUM(I))
CURVE NUMBER
(((FLOAT(NUM(I)))*(GCN2(I,J)))-((GCN1(I,J))*
FLOAT(NUM(I)))*((FLOAT(NUM(I)))-1.0)))
ANDARD DEVIATIONS FOR GCN VALUES
,J)/(SQRT(FLOAT(NUM(I))))
ANDARD ERRORS FOR GCN VALUES

WTH CURVE NUMBERS AS AVERAGES OF TOTAL FOR
BY NUMBER OF REPLICATES

J, SD, SE
```

```
      PROGRAM    TST          73/73   TS                                FTN 4.6+420

                 DO 91 I=1,18
                   WRITE(6,102) I,NUM(I),(GCN(I,J),J=1,7)
           102     FORMAT(2X,2(I3,5X),F8.2,7(F8.2,2X),/)
                   WRITE(6,104)(SD3(I,J),J=1,7)
           104     FORMAT(18X,5HSD3= ,7(F8.2,2X),/)
                   WRITE(6,106)(SE1(I,J),J=1,7)
           106     FORMAT(18X,5HSE1= ,7(F8.2,2X),///)
           91      CONTINUE
                 DO 41 I=1,10
                   JJ=1
                   DO 42 J=1,7
                   I1=I+19
                   IF(I1.EQ.29) I1=12
                   IF(JJ.EQ.4) JJ=JJ+1
                   IF(JJ.EQ.8) JJ=JJ+1
                 DEFINE SCALE OF PLOT
                   CALL SCALE(0.0,4.5,0.75,9.25)
                 PLOT GCN VALUES FOR SPORES OF ASCUS PLUS PARENTS
                   CALL PLOTPT(GCN(I,J),FLOAT(JJ),I1)
                 FIRST PLOT CONTAINS PARENTS, 1 AND 2, AND THE EIGHT
                 SPORES OF ONE ASCUS, A THRU H
                   JJ=JJ+1
           42      CONTINUE
           41    CONTINUE
                 PLOT GCN VALUES FOR SPORES OF ASCUS PLUS PARENTS
                 PLOT ON OUTPUT
                   CALL OUTPLT
                   IG=IG+1
                 IG FOR COUNTING
                   JJ=1
                   DO 81 J=1,7
                     IF(JJ.EQ.4) JJ=JJ+1
                     IF(JJ.EQ.8) JJ=JJ+1
                 DEFINE SCALE OF PLOT
                     CALL SCALE(0.0,4.5,0.75,9.25)
                 PLOT FIRST PARENT FOR THE SECOND PLOT
                   CALL PLOTPT(GCN(1,J),FLOAT(JJ),11)
                   JJ=JJ+1
           81    CONTINUE
                 DO 91 I=10,18
                   JJ=1
                     DO 92 J=1,7
                       I1=I+19
                       IF(I1.EQ.29)I1=12
                 I2 FOR COUNTING
                       IF(JJ.EQ.4) JJ=JJ+1
```



```
      PROGRAM    TST          73/73   TS                                FTN 4.6+420

                       IF(JJ.EQ.8) JJ=JJ+1
                       CALL SCALE(0.0,4.5,0.75,9.25)
                       CALL PLOTPT(GCN(I,J),FLOAT(JJ),I1)
                 SECOND PLOT CONTAINS PARENTS, 1 AND 2, AND THE EIGHT SPORES
                 ASCUS, J THRU Q
                     JJ=JJ+1
           92        CONTINUE
```

```
(GCN(I,J),J=1,7)
,7(F8.2,2X),/)
J=1,7)
8.2,2X),/)
,J=1,7)
8.2,2X),///)
```

```
.75,9.25)
F ASCUS PLUS PARENTS
,FLOAT(JJ),I1)
 1 AND 2, AND THE EIGHT
H
```

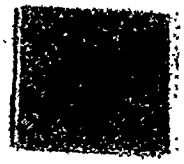
```
F ASCUS PLUS PARENTS
```

```
.75,9.25)
COND PLOT
LOAT(JJ),11)
```



```
.75,9.25)
,FLOAT(JJ),I1)
, 1 AND 2, AND THE EIGHT SPORES OF OTHER
```

```
                IF(JJ.EQ.8) JJ=JJ+1
                CALL SCALE(0.0,4.5,0.75,9.25)
                CALL PLOTPT(GCN(I,J),FLOAT(JJ),I1)
C SECOND PLOT CONTAINS PARENTS, 1 AND 2, AND THE EIGHT SPORES OF
C ASCUS, J THRU Q
              JJ=JJ+1
92            CONTINUE
91          CONTINUE
C
            CALL OUTPLT
            IG=IG+1
            M=0
            DO 95 I=2,16,2
              M=M+1
              DO 962 J=1,7
                I1=I
                IF(I1.GE.10) I1=I+1
                I2=I1+1
                NUM2(M)=NUM(I1)+NUM(I2)
                GCNS(M,J)=(((GCN(I1,J)*FLOAT(NUM(I1)))+(GCN(I1+1,J)*
     8FLOAT(NUM(I1+1)))/((FLOAT(NUM(I1)))+(FLOAT(NUM(I1+1))))
C GCNS CONTAINS GCN VALUES FOR SPORE PAIRS
962           CONTINUE
95            CONTINUE
C
C FIRST ASCUS PLOT FOR LUMPED SPORE PAIRS
            DO 96 M=1,4
            JJ=1
              DO 961 J=1,7
                IF(JJ.EQ.4) JJ=JJ+1
                IF(JJ.EQ.8) JJ=JJ+1
                I1=M+2
                CALL SCALE(0.0,4.5,0.75,9.25)
                CALL PLOTPT(GCNS(M,J),FLOAT(JJ),I1)
                JJ=JJ+1
961           CONTINUE
96          CONTINUE
C
C GO TO ADD PARENTS IF NOT ALREADY INCLUDED IN PLOT
            IF(M.GE.4) GO TO 99
            M=5
C SECOND ASCUS PLOT FOR LUMPED SPORE PAIRS
            DO 97 M=5,8
            JJ=1
              DO 971 J=1,7
                I1=M+25
                IF(JJ.EQ.4) JJ=JJ+1
                IF(JJ.EQ.8) JJ=JJ+1
                CALL SCALE(0.0,4.5,0.75,9.25)
                CALL PLOTPT(GCNS(M,J),FLOAT(JJ),I1)
              JJ=JJ+1
971           CONTINUE
97            CONTINUE
```



```
PROGRAM   TST        73/73   TS                                FTN 4.6+420
C
99          DO 98 I=1,10,9
            JJ=1
              DO 981 J=1,7
                I1=I+10
                IF(JJ.EQ.4) JJ=JJ+1
                IF(JJ.EQ.8) JJ=JJ+1
               IF(I1.GT.11) I1=12
                CALL SCALE(0.0,4.5,0.75,9.25)
C PLOT PARENTS FOR THE THIRD AND FOURTH PLOTS
                CALL PLOTPT(GCN(I,J),FLOAT(JJ),I1)
              JJ=JJ+1
981           CONTINUE
98            CONTINUE
C
C PRINT PLOT IN OUTPUT
            CALL OUTPLT
C THIRD PLOT CONTAINS PARENTS, 1 AND 2 , AND THE FOUR SPORE PAI
C OF ONE ASCUS, A THRU D
C FOURTH PLOT CONTAINS PARENTS, 1 AND 2, AND THE FOUR SPORE PAI
C OF OTHER ASCUS, J THRU M
```

```
.75,9.25)
,FLOAT(JJ),I1)
; 1 AND 2, AND THE EIGHT SPORES OF OTHER
```

```
I2)
J)*FLOAT(NUM(I1)))+(SCN(I1+1,J)*
AT(NUM(I1)))+(FLOAT(NUM(I1+1))))
SPORE PAIRS
```

```
SPORE PAIRS
```

```
.75,9.25)
,FLOAT(JJ),I1)
```

```
READY INCLUDED IN PLOT
```

```
R SPORE PAIRS
```

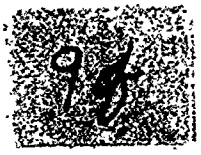
```
0.75,9.25)
J),FLOAT(JJ),I1)
```



```
1)
0.75,9.25)
 AND FOURTH PLOTS
J),FLOAT(JJ),I1)
```

```
S, 1 AND 2 , AND THE FOUR SPORE PAIRS
TS, 1 AND 2, AND THE FOUR SPORE PAIRS
```

```
220           IF(I1.GT.14) I1=12
              CALL SCALE(0.0,4.5,0.75,9.25)
C PLOT PARENTS FOR THE THIRD AND FOURTH PLOTS
              CALL PLOTPT(GCN(I,J),FLOAT(JJ),I1)
            JJ=JJ+1
225       981  CONTINUE
          98     CONTINUE
C
C PRINT PLOT IN OUTPUT
            CALL OUTPLT
C THIRD PLOT CONTAINS PARENTS, 1 AND 2 , AND THE FOUR SPORE PAI
C OF ONE ASCUS, A THRU D
C FOURTH PLOT CONTAINS PARENTS, 1 AND 2, AND THE FOUR SPORE PAI
C OF OTHER ASCUS, J THRU M
            IG=IG+1
C GO BACK AND PLOT FOURTH GRAPH IF NOT DONE
            IF(IG.LE.3) GO TO 94
C PRINTOUT OF M , NUM2, GCNS, (FOR SPORE PAIRS)
            DO 983 M=1,8
C M COUNTS SPORE PAIRS
              WRITE(6,984) M, NUM2(M), (GCNS(M,J),J=1,7)
          984   FORMAT(2X,2(I3,5X),5X,7(F8.2,2X),/)
          983 CONTINUE
C
C START TOTAL GROWTH NUMBERS CALCULATIONS (TGN)
            DO 201 K=1,2
              DO 202 I=1,18
                DO 204 J=1,7
                  IF(IDATA(I,J,K)-9) 206,202,202
          206     TGN1(I,K)=TGN1(I,K)+FLOAT(IDATA(I,J,K))
C TGN1 CONTAINS TOTAL GROWTH NUMBERS FOR EACH INOCULUM
          204       CONTINUE
          202   CONTINUE
          201   CONTINUE
C
            DO 208 I=1,18
              DO 208 K=1,20
                TGN2(I)=TGN2(I)+TGN1(I,K)
                TGN3(I)=TGN3(I)+((TGN1(I,K))**2)
C TGN2 NOW CONTAINS SUMMED TGNS FOR EACH INOCULUM
C TGN3 NOW CONTAINS THE SUM OF SQUARES OF DATA FOR EACH I
          208 CONTINUE
C
            DO 212 I=1,18
              IF(NUM(I).EQ.0) GO TO 213
C CALCULATE MEAN TGN FOR STRAIN
```



```
PROGRAM    TST        73/73   TS                                    FTN 4.6+420

              AMEAN(I)=TGN2(I)/FLOAT(NUM(I))
C STANDARD DEVIATION CALCULATION FOR TGN VALUES
              SD(I)=SQRT((((FLOAT(NUM(I)))*(TGN3(I)))-((TGN2(I))*(TGN
             1((FLOAT(NUM(I)))*((FLOAT(NUM(I)))-1.0)))
C STANDARD ERROR CALCULATION FOR TGN VALUES
              SE(I)=SD(I)/(SQRT(FLOAT(NUM(I))))
              GO TO 215
          213   SD(I)=0.0
                AMEAN(I)=0.0
                SE(I)=0.0
C PRINTOUT I, NUM, AMEAN(MEAN TGN VALUE), SD, SE
          215   WRITE(6,214) I,NUM(I),AMEAN(I),SD(I),SE(I)
          214   FORMAT(1HO,2HI=,I3,3X,4HNUM=,I4,3X,5HMEAN=,F5.2,3X,3HSD
               23X,3HSE=,F7.2)
          212   CONTINUE
C
            DO 500 I=1,18
C TITLE PRINTOUT
              WRITE(6,502)
          502   FORMAT(1H ,30X,13HUNSORTED DATA,//)
C FORMAT TO PRINTOUT ALL INOCULUM TGN VALUES
              WRITE(6,504) I,(TGN1(I,K),K=1,20)
          504   FORMAT(1X,3HI= ,I3,5X,20(1X,F5.1),/)
          500   CONTINUE
C
              M=1
            DO 600 I=2,16,2
              I1=I
              IF(I1.GE.10) I1=I1+1
                                                                    1
```

```
12
0,4.5,0.75,3.25)
THIRD AND FOURTH PLOTS
GN(I,J),FLOAT(JJ),I=1)



ARENTS, 1 AND 2 , AND THE FOUR SPORE PAIRS
0
PARENTS, 1 AND 2, AND THE FOUR SPORE PAIRS
U 4

TH GRAPH IF NOT DONE
0 94
GONS, (FOR SPORE PAIRS)


, NUM2(M), (GONS(M,J),J=1,7)
5X),5X,7(F8.2,2X),/)

UMBER CALCULATIONS (TGN)


I,J,K)-9)206,2?2,206
TGN1(I,K)=FLOAT(IDATA(I,J,K))
GROWTH NUMBERS FOR EACH INOCULUM




(I)+TGN1(I,K)
(I)+((TGN1(I,K))**2)
UMMED TGNS FOR EACH INOCULUM
HE SUM OF SQUARES OF DATA FOR EACH I


0.0) GO TO 213
OR STRAIN

S                              FTN 4.6+420      09/19/76  14.05.40      PAGE   6

(I)/FLOAT(NUM(I))
CALCULATION FOR TGN VALUES
(FLOAT(NUM(I)))*(TGN3(I)))-((TGN2(I))*(TGN2(I)))/
)*((FLOAT(NUM(I)))-1.0)))
ULATION FOR TGN VALUES
SQRT(FLOAT(NUM(I))))

0

EAN(MEAN TGN VALUE), SD, SE
,NUM(I),AMEAN(I),SD(I),SE(I)
I=,I3,3X,4HNUM=,I4,3X,5HMEAN=,F5.2,3X,3HSD=,F7.2,

X,13HUNSORTED DATA,//)
ALL INOCULUM TGN VALUES
,(TGN1(I,K),K=1,20)
I= ,I3,5X,20(1X,F5.1),/)


I1=I1+1

                                    TGN VAL
```

```
  500    CONTINUE
         M=1
         DO 600 I=2,16,2
         I1=I
         IF(I1.GE.10) I1=I1+1
         I2=I1+1
C STUDT CALCULATIONS ARE FOR VARIOUS COMPARISONS BETWEEN STRAI
C USING THE STUDENT'S T STATISTIC
C STUDT1(I1) COMPARES TGN FOR PAIRS
         STUDT1(M)=((ABS(AMEAN(I1)-AMEAN(I2)))/(SQRT((SD(I1)**
        2(SD(I2)**2))))
C STUDTP EQUALS TGN SPORE VS TGN PARENT ONE COMPARISON
         STUDTP(M)=((ABS(AMEAN(1)-AMEAN(I1)))/(SQRT((SD(1)**2)+
        4(SD(I1)**2))))
C STUDT0(I1) EQUALS TGN SPORE VS TGN PARENT TWO COMPARISON
         STUDT0(M) =((ABS(AMEAN(10)-AMEAN(I1)))/(SQRT((SD(10)**
        5(SD(I1)**2))))
CSTUDT2 COMPARES SECOND MEMBER OF PAIR WITH PARENT ONE
         STUDT2(M)=((ABS(AMEAN(1)-AMEAN(I2)))/(SQRT((SD(1)**2)
        6(SD(I2)**2))))
C STUDT3 COMPARES SECOND MEMBER OF PAIR WITH PARENT TWO
         STUDT3(M)=((ABS(AMEAN(10)-AMEAN(I2)))/(SQRT((SD(10)**2
        7(SD(I2)**2))))
C NUM2(M) IS TOTAL INOCULAE FOR BOTH MEMBERS OF PAIRS
         NUM2(M)=NUM(I1)+NUM(I2)
C AMEAN2(M) IS MEAN FOR TOTAL PAIR TESTS
         AMEAN2(M)=((AMEAN(I1)*NUM(I1))+(AMEAN(I2)*NUM(I2)))/NUM
C TGN4(M) IS TOTAL OF OBS FOR PAIRS
         TGN4(M)=TGN2(I1)+TGN2(I2)
```


```
      PROGRAM    TST        73/73    TS                          FTN 4.6+420

C TGN5(M) IS TOTAL OF OBS SQUARED OF PAIRS
         TGN5(M)=TGN3(I1)+TGN3(I2)
C SD2 (M) GIVES SD FOR SPORE PAIR DATA LUMPED
         SD2(M)=SQRT((((FLOAT(NUM2(M)))*(TGN5(M)))-((TGN4(M))*
        8(TGN4(M)))/(((FLOAT(NUM2(M)))*((FLOAT(NUM2(M)))-1.0)))
C LUMPED PAIR VS FIRST PARENT
         STUDT4(M)=((ABS(AMEAN(1)-AMEAN2(M)))/(SQRT((SD(1)**2)+
        F(SD2(M)**2))))
C LUMPED PAIR DATA VS SECOND PARENT
         STUDT5(M)=((ABS(AMEAN(10)-AMEAN2(M)))/(SQRT((SD(10)**2)
        G(SD2(M)**2))))
         M=M+1
  600    CONTINUE
C
         DO 605 M=1,8
         WRITE(6,610) M, STUDT1(M), STUDTP(M), STUDT0(M), STUDT2
        ASTUDT3(M), NUM2(M), AMEAN2(M)
  610    FORMAT(1H0,3HM= ,I3,2X,8HSTUDT1= ,F5.2,2X,8HSTUDTP= ,F
        B8HSTUDT0= ,F5.2,2X,8HSTUDT2= ,F5.2,2X,8HSTUDT3= ,F5.2,2X
        D6HNUM2= ,I3,2X,8HAMEAN2= ,F5.2,/)
         WRITE(6,611) TGN4(M), TGN5(M), SD2(M),STUDT4(M), STUDT
  611    FORMAT(1H0,6HTGN4= ,F8.1,2X,6HTGN5= ,F8.1,2X,5HSD2= ,F
        H  8HSTUDT4= ,F5.2,2X,8HSTUDT5= ,F5.2,///)
C PRINTOUT ALL VALUES NAMED
  605    CONTINUE
C
         CALL READX (X)
         IF(X.GE.1.0) GO TO 999
         STOP
         END
```

--ENTRY POINTS--

```
        3B   INPUT=          439   OUTPUT=          3B   T
      112B   TST
```

--EXTERNALS--

```
FINRPV.   INPCI.   INPCR.   OUTCI.   OUTCR.   OUTPLT   P
SCALE     SQRT.    STOP.
```

--STATEMENT LABELS--

```
2X,13HUNSORTED DATA,//)
 ALL INOCULUM TGN VALUES
I,(TGN1(I,K),K=1,20)
I= ,I3,2X,20(1X,F5.1),/)


2

 I1=I1+1

 ARE FOR VARIOUS COMPARISONS BETWEEN STRAIN TGN VALUES
S T STATISTIC
S TGN FOR PAIRS
(ABS(AMEAN(I1)-AMEAN(I2)))/(SQRT((SD(I1)**2)+

SPORE VS TGN PARENT ONE COMPARISON
(ABS(AMEAN(1)-AMEAN(I1)))/(SQRT((SD(1)**2)+

LS TGN SPORE VS TGN PARENT TWO COMPARISON
((ABS(AMEAN(10)-AMEAN(I1)))/(SQRT((SD(10)**2)+

ONND MEMBER OF PAIR WITH PARENT ONE
((ABS(AMEAN(1)-AMEAN(I2)))/(SQRT((SD(1)**2)+

S SECOND MEMBER OF PAIR WITH PARENT TWO
((ABS(AMEAN(10)-AMEAN(I2)))/(SQRT((SD(10)**2)+

INOCULAE FOR BOTH MEMBERS OF PAIRS
(I1)+NUM(I2)
 FOR TOTAL PAIR TESTS
(AMEAN(I1)*NUM(I1))+(AMEAN(I2)*NUM(I2)))/NUM2(M)
OF OBS FOR PAIRS
)(I1)+TGN2(I2)
```



```
OF OBS SQUARED OF PAIRS
3(I1)+TGN3(I2)
FOR SPORE PAIR DATA LUMPED
((((FLOAT(NUM2(M)))*(TGN5(M)))-((TGN4(M))*
((FLOAT(NUM2(M)))*((FLOAT(NUM2(M)))-1.0)))
RST PARENT
(ABS(AMEAN(1)-AMEAN2(M)))/(SQRT((SD(1)**2)+
)))
VS SECOND PARENT
(ABS(AMEAN(10)-AMEAN2(M)))/(SQRT((SD(10)**2)+
)


) M, STUDT1(M), STUDTP(M), STUDT0(M), STUDT2(M),
M2(M), AMEAN2(M)
3HM= ,I3,2X,8HSTUDT1= ,F5.2,2X,8HSTUDTP= ,F5.2,2X,
.2,2X,8HSTUDT2= ,F5.2,2X,8HSTUDT3= ,F5.2,2X,
X,8HAMEAN2= ,F5.2,/)
) TGN4(M), TGN5(M), SD2(M), STUDT4(M), STUDT5(M)
6HTGN4= ,F8.1,2X,6HTGN5= ,F8.1,2X,5HSD2= ,F5.2,2X,
F5.2,2X,8HSTUDT5= ,F5.2,///)
IES NAMED

)
GO TO 999
```

--ENTRY POINTS--

[illegible] INPUT [illegible] 430 OUTPUT= [illegible]

--EXTERNALS--

FTNRPV. INPCI. INPCR. OUTCI. OUTCR. UTPLT PL
SCALE SQRT. STOP.

--STATEMENT LABELS--

.1	ID	2518	.11	B	3558	.12		
.15	D	3528	.16		3208	.19	F	
.21	D	4468	.31	ID	08	.41	ID	
.49	ID	08	.51	ID	08	.55	ID	
.71	ID	08	.81	ID	08	.91	ID	
.94		10328	.95	ID	08	.96	ID	
.98	ID	08	.99		11008	.100	F	

PROGRAM TST 73/73 TS FTN 4.6+420

--STATEMENT LABELS--

.104	F	16378	.106	F	16438	.201	D	
.204	D	12318	.205		12158	.208	ID	
.213		13158	.214	F	16538	.215		
.502	F	16628	.504	F	16668	.600	ID	
.610	F	16728	.611	F	17108	.961	ID	
.971	ID	08	.981	ID	08	.983	ID	
.999		1268						

--VARIABLE MAP--

ABS	R		INTRINSIC			AMEAN	R	74428			
AMEAN2	R	77128		8		FLOAT	R				IN
FTNRPV.	-		EXTERNAL.			GCN	R	20558			
GCNS	R	73178		56		GCN1	R	74748			
GCN2	R	105448		126		I	I	U	72048		
IDATA	I	U 22548		2520		IG	I		22538		
INPCI.	-		EXTERNAL.			INPCR.	-				EX
INPUT=	-	38	ENTRY			I1	I		113568		
I2	I	72748				J	I	U	72058		
JJ	I	74418				K	I	U	72308		
M	I	72638				NUM	I		74178		
NUM2	I	74078		8		OUTCI.	-				EX
OUTCR.	-		EXTERNAL.			OUTPLT	R				SU
OUTPUT=	-	438	ENTRY			PLOTPT	R				SU
READX	R		SUBROUTINE			SCALE	R				SU
SD	R	72758		18		SD2	R	105348			
SD3	R	107428		126		SE	R	104728			
SE1	R	111408		126		SQRT.	R				R.
STOP.	-		EXTERNAL.			STUDTP	R	74648			
STUDT0	R	105148		8		STUDT1	R	76728			
STUDT2	R	105248		8		STUDT3	R	77028			
STUDT4	R	113368		8		STUDT5	R	113468			
TAPE5=	-	38	ENTRY			TAPE6=	-	439			EN
TGN1	R	77228		360		TGN2	R	72068			
TGN3	R	72318		18		TGN4	R	72538			
TGN5	R	72648		8		TST	-	1128			EN
X	R	113578									

15434B PROGRAM-UNIT LENGTH 106 SYMBOLS

440008 CM STORAGE USED 2.347 SECONDS

[redacted]

? OUTPUT= ?B TAPE6= 43B TAPE6=

OUTCI. OUTCP. OUTPLT PLOTPT READX

D	355B	.12		300B	.14		311B
	320B	.19	F	1626B	.20		436B
ID	0B	.41	ID	0B	.42	ID	0B
ID	0B	.55	ID	0B	.61	ID	0B
ID	0B	.91	ID	0B	.92	ID	0B
ID	0B	.96	ID	0B	.97	ID	0B
	1100B	.100	F	1631B	.102	F	1633B

F	1643B	.201	D	1237B	.202	D	1234B
	1215B	.208	ID	0B	.212	ID	0B
F	1653B	.215		1324B	.508	ID	0B
F	1666B	.600	ID	0B	.605	ID	0B
F	1710B	.961	ID	0B	.962	ID	0B
ID	0B	.983	ID	0B	.984	F	1647B

AMEAN	R		7442B		18
FLOAT	R			INTRINSIC	
GCN	R		2055B		126
GCN1	R		7474B		125
I	I	U	7204B		
IG	I		2253B		
INPCR.	-			EXTERNAL.	
I1	I		11356B		
J	I	U	7205B		
K	I	U	7230B		
NUM	I		7417B		18
OUTCI.	-			EXTERNAL.	
OUTPLT	R			SUBROUTINE	
PLOTPT	R			SUBROUTINE	
SCALE	R			SUBROUTINE	
SD2	R		10534B		8
SE	R		10472B		18
SQRT.	R			B.E.F.	
STUDTP	R		7464B		8
STUDT1	R		7672B		8
STUDT3	R		7702B		8
STUDT5	R		11346B		8
TAPE6=	-		43B	ENTRY	
TGN2	R		7206B		18
TGN4	R		7253B		8
TST	-		112B	ENTRY	

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NOS

154748 PROGRAM-UNIT LENGTH 106 SYMBOLS

440008 CM STORAGE USED 2.347 SECONDS

[redacted]

LOAD MAP - TST CYBER LOADER 1.0-P1

FWA OF THE LOAD 101
LWA+1 OF THE LOAD 35663

TRANSFER ADDRESS -- TST 213

PROGRAM AND BLOCK ASSIGNMENTS.

BLOCK	ADDRESS	LENGTH	FILE	DATE	PROCSSR	VE	LEVEL	HARD
TST	101	15434	LGO	09/19/76	FTN	4.6	420	6464
/STP.END/	15535	1						
/FCL.C./	15536	23						
/ACSQACS/	15561	3						
/Q8.IO./	15564	133						
Q8NTRY=	15717	0	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
COMIQ=	15717	64	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
FECMSK=	16003	41	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
FLTOUT=	16044	311	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
FORSYS=	16355	602	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
GETFIT=	17157	42	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
KRAKER=	17221	406	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
OUTC=	17627	172	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
SYSAID=	20021	1	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
/A0B.RM/	20022	10						
/CON.RM/	20032	6						
ERR.RM	20040	404	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
CHWR.SQ	20444	7	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
OSUB.RM	20453	65	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/JMPS.RM/	20540	11						
/OPEN.FO/	20551	7						
OPEN.SQ	20560	262	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
OPEX.SQ	21042	14	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/PUT.RT/	21056	11						
RLEQ.RM	21067	42	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/CLSF.FO/	21131	7						
CLSF.SQ	21140	131	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/CLSV.FO/	21271	7						
CLSV.SQ	21300	123	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/REW.FO/	21423	7						
REW.SQ	21432	31	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/TERM.RM/	21463	1						
/GET.FO/	21464	7						
/GET.BT/	21473	5						
/GET.RT/	21500	11						
GET.SQ	21511	1033	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
Z.SQ	22544	101	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
FSU.SQ	22645	106	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
FLTIN=	22753	154	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
FMTAP=	23127	352	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
FORUTL=	23501	16	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
FTNRPV=	23517	145	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
INCOM=	23664	276	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
INPC=	24162	160	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
KODER=	24342	456	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	

DATE	PROCSSR	VER	LEVEL	HARDWARE	COMMENTS
09/19/76	FTN	4.6	420	6464 I	PROGRAM TS
05/31/76	COMPASS	3.	3-420		FCL INITILALIZATION ROUTINE.
05/31/76	COMPASS	3.	3-420		COMMON CODED I/O ROUTINES AND CONSTANTS.
05/31/76	COMPASS	3.	3-420		INITIALIZE CONSTANTS.
05/31/76	COMPASS	3.	3-420		COMMON FLOATING OUTPUT CODE
05/31/76	COMPASS	3.	3-420		FORTRAN OBJECT LIBRARY UTILITIES.
05/31/76	COMPASS	3.	3-420		LOCATE AN FIT GIVEN A FILE NAME.
05/31/76	COMPASS	3.	3-420		PROCESS FORMATTED FORTRAN INPUT.
05/31/76	COMPASS	3.	3-420		FORMATTED WRITE FORTRAN RECORD.
05/31/76	COMPASS	3.	3-420		LINK BETWEEN SYS=AID AND INITIALIZATION CODE.
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
02/20/76	COMPASS	3.	75115		
05/31/76	COMPASS	3.	3-420		COMMON FLOATING INPUT CONVERTER.
05/31/76	COMPASS	3.	3-420		CRACK APLIST AND FORMAT FOR KODER/KRAKER.
05/31/76	COMPASS	3.	3-420		FCL MISC. UTILITIES.
05/31/76	COMPASS	3.	3-420		FORTRAN REPRIEVE.
05/31/76	COMPASS	3.	3-420		COMMON INPUT FORMATTING CODE
05/31/76	COMPASS	3.	3-420		FORMATTED READ FORTRAN RECORD.
05/31/76	COMPASS	3.	3-420		OUTPUT FORMAT INTERPRETER.

FLTIN=	22753	154	SL-FORTRAN	05/31/76	COMPASS	3.	3-420
FMTAP=	23127	252	SL-FORTRAN	05/31/76	COMPASS	3.	3-420
FORUTL=	23501	116	SL-FORTRAN	05/31/76	COMPASS	3.	3-420
FTNRPV=	23517	145	SL-FORTRAN	05/31/76	COMPASS	3.	3-420
INCOM=	23664	376	SL-FORTRAN	05/31/76	COMPASS	3.	3-420
INPC=	24152	160	SL-FORTRAN	05/31/76	COMPASS	3.	3-420
KONFP=	24342	456	SL-FORTRAN	05/31/76	COMPASS	3.	3-420

LOAD MAP - TST CYBER LOADER 1.0-PFI

OUTCOM=	25020	154	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
SQRT	25174	43	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
SYS=1ST	25237	62	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
CIO.RM	25321	40	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
MOVE.RM	25361	64	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
MCT.RM	25445	227	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/MEMC.RM/	25674	3						
/OPES.FO/	25677	1						
OPEN.RM	25700	231	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/PUT.FO/	26131	7						
PUT.SQ	26140	1360	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
WAP.SQ	27520	260	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
CLSF.RM	28000	23	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
BTPT.SQ	30023	114	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
WEOX.SQ	30137	142	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
/SKFL.FO/	30301	7						
SKFL.SQ	30310	47	SL-FORTRAN	02/20/76	COMPASS	3.	75115	
SYS.RM	30357	37	SL-SYSIO	07/06/76	COMPASS	3.	75115	
ACS4MTP	30416	213	SL-MMUFTNL	05/28/76	COMPASS	3.	3-420	
J5001	30631	307	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
OUTPLT	31140	17	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
SCALE	31157	27	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
J9002	31206	3140	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
PLOTPT	34346	24	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
SCALE2	34372	257	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
READCH	34651	262	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
READX	35133	175	SL-MMUFTNL	05/28/76	FTN	4.6	420	6464
RECOVR	35330	166	SL-NUCLEUS	07/06/76	COMPASS	3.	75115	
GOTOER=	35516	14	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
ALOG	35532	73	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
XTOI*	35625	26	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	
XTOI=	35653	10	SL-FORTRAN	05/31/76	COMPASS	3.	3-420	

4.045 CP SECONDS 475778 CM STORAGE USED

[redacted]

ASCI

1	20	0.00	.10	.65	1.15	1.2.	1.25
	SD3=	0.00	.31	.49	.37	.41	.44
	SE1=	0.00	.07	.11	.08	.0?	.10

```
AN  05/31/76 COMPASS  3. 3-420            CRACK APLIST AND FORMAT FOR KODER/KRAKER.
AN  05/31/76 COMPASS  3. 3-420            FCL MISC. UTILITIES.
AN  05/31/76 COMPASS  3. 3-420            FORTRAN REPRIEVE.
AN  05/31/76 COMPASS  3. 3-420            COMMON INPUT FORMATTING CODE
AN  05/31/76 COMPASS  3. 3-420            FORMATTED READ FORTRAN RECORD.
AN  05/31/76 COMPASS  3. 3-420            OUTPUT FORMAT INTERPRETER.
AN  05/31/76 COMPASS  3. 3-420
```



```
AN  05/31/76 COMPASS  3. 3-420            COMMON OUTPUT CODE
AN  05/31/76 COMPASS  3. 3-420            COMPUTE THE SQUARE ROOT OF X. OPT=ALL.
AN  05/31/76 COMPASS  3. 3-420            MATH LIBRARY LINK TO ERROR MESSAGE PROCESSOR.
AN  02/20/76 COMPASS  3. 75115
AN  02/20/76 COMPASS  3. 75115
AN  02/20/76 COMPASS  3. 75115

AN  02/20/76 COMPASS  3. 75115

AN  02/20/76 COMPASS  3. 75115
AN  02/20/76 COMPASS  3. 75115
AN  02/20/76 COMPASS  3. 75115
AN  02/20/76 COMPASS  3. 75115
AN  02/20/76 COMPASS  3. 75115

AN  02/20/76 COMPASS  3. 75115
)   07/06/76 COMPASS  3. 75115            PROCESS SYSTEM REQUEST.
NL  05/28/76 COMPASS  3. 3-420
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
NL  05/28/76 FTN      4.6 420   6464 I    OPT=2 ROUND=+-*/ TRACE
US  07/06/76 COMPASS  3. 75115            REPRIEVE INTERFACE
RAN 05/31/76 COMPASS  3. 3-420            COMPUTED GO TO ERROR PROCESSOR.
RAN 05/31/76 COMPASS  3. 3-420            COMPUTE COMMON AND NATURAL LOGARITHMS. OPT=ALL.
RAN 05/31/76 COMPASS  3. 3-420            REAL BASE TO INTEGER POWER.
RAN 05/31/76 COMPASS  3. 3-420            REAL TO INTEGER EXPONENTIATION.

75778 CM STORAGE USED                96 TABLE MOVES
```

.65	1.15	1.20	1.25	1.25
.49	.37	.41	.44	.44
.11	.08	.09	.10	.10

1	20		0.00	.10	.65	1.15	1.2	1.25
		SD3=	0.00	.31	.49	.37	.41	.44
		SE1=	0.00	.07	.11	.08	.0	.10
2	20		0.00	.15	.30	.55	.6	.90
		SD3=	0.00	.37	.47	.69	.75	.64
		SE1=	0.00	.08	.11	.15	.17	.14
3	20		0.00	.30	.80	1.30	1.35	1.50
		SD3=	0.00	.47	.70	.47	.49	.51
		SE1=	0.00	.11	.16	.11	.11	.11
4	20		.10	.25	.80	1.15	1.19	1.20
		SD3=	.45	.55	.52	.37	.37	.41
		SE1=	.10	.12	.12	.08	.08	.09
5	20		0.00	.10	.60	.95	1.00	1.05
		SD3=	0.00	.31	.50	.22	0.00	.22
		SE1=	0.00	.07	.11	.05	0.00	.05
6	20		.05	1.05	1.45	1.90	1.90	1.95
		SD3=	.22	.39	.51	.31	.31	.22
		SE1=	.05	.09	.11	.07	.07	.05
7	20		.10	1.05	1.80	2.00	2.00	2.05
		SD3=	.45	.39	.41	.32	.32	.22
		SE1=	.10	.09	.09	.07	.07	.05
8	20		.20	1.40	1.95	2.15	2.50	2.60
		SD3=	.52	.60	.22	.37	.61	.60
		SE1=	.12	.13	.05	.08	.14	.13
9	20		.45	1.55	1.75	1.95	2.15	2.35
		SD3=	.69	.60	.44	.39	.37	.49
		SE1=	.15	.14	.10	.09	.08	.11
	20		1.25	1.75	1.95	1.95	1.95	2.00

	0.00	.10	.65	1.15	1.20	1.25	1.25
SD3=	0.00	.31	.49	.37	.41	.44	.44
SE1=	0.00	.07	.11	.08	.09	.10	.10
	0.00	.15	.30	.55	.60	.90	1.10
SD3=	0.00	.37	.47	.69	.75	.64	.55
SE1=	0.00	.09	.11	.15	.17	.14	.12
	0.00	.30	.80	1.30	1.35	1.50	1.65
SD3=	0.00	.47	.70	.47	.43	.51	.49
SE1=	0.00	.11	.16	.11	.11	.11	.11
	.10	.25	.80	1.15	1.15	1.20	1.45
SD3=	.45	.55	.52	.37	.37	.41	.51
SE1=	.10	.12	.12	.08	.08	.09	.11
	0.00	.15	.60	.95	1.00	1.05	1.10
SD3=	0.00	.31	.50	.22	0.00	.22	.31
SE1=	0.00	.07	.11	.05	0.00	.05	.07
	.05	1.05	1.45	1.90	1.90	1.95	2.00
SD3=	.22	.39	.51	.31	.31	.22	.32
SE1=	.05	.09	.11	.07	.07	.05	.07
	.10	1.05	1.80	2.00	2.00	2.05	2.15
SD3=	.45	.39	.41	.32	.32	.22	.37
SE1=	.10	.09	.09	.07	.07	.05	.08
	.20	1.40	1.95	2.15	2.50	2.60	3.15
SD3=	.52	.60	.22	.37	.61	.60	.59
SE1=	.12	.13	.05	.08	.14	.13	.13
	.45	1.55	1.75	1.95	2.15	2.35	2.65
SD3=	.69	.60	.44	.39	.37	.49	.67
SE1=	.15	.14	.10	.09	.09	.11	.15
	1.25	1.75	1.95	1.95	1.95	2.00	2.00
SD3=	.79	.55	.22	.22	.22	0.00	0.00
SE1=	.18	.12	.05	.05	.05	0.00	0.00

		SD3=	.69	.60	.44	.39	.37	.49
		SE1=	.15	.14	.10	.09	.08	.11
10	20		1.25	1.75	1.95	1.95	1.95	2.00
		SD3=	.79	.55	.22	.22	.22	0.00
		SE1=	.18	.12	.05	.05	.05	0.00
11	20		0.00	0.00	0.00	0.00	0.00	0.00
		SD3=	0.00	0.00	0.00	0.00	0.00	0.00
		SE1=	0.00	0.00	0.00	0.00	0.00	0.00
12	20		0.00	0.00	0.00	0.00	0.00	0.00
		SD3=	0.00	0.00	0.00	0.00	0.00	0.00
		SE1=	0.00	0.00	0.00	0.00	0.00	0.00
13	20		.10	1.15	1.90	1.95	2.00	2.00
		SD3=	.31	.59	.31	.22	0.00	0.00
		SE1=	.07	.13	.07	.05	0.00	0.00
14	20		.10	.85	1.55	1.90	2.00	2.00
		SD3=	.31	.37	.51	.31	0.00	0.00
		SE1=	.07	.08	.11	.07	0.00	0.00
15	20		.05	.85	1.00	1.10	1.20	1.25
		SD3=	.22	.37	0.00	.31	.41	.44
		SE1=	.05	.08	0.00	.07	.09	.10
16	20		.05	.85	1.10	1.30	1.35	1.45
		SD3=	.22	.37	.31	.47	.47	.51
		SE1=	.05	.08	.07	.11	.11	.11
17	20		1.65	2.35	2.55	3.25	3.35	3.50
		SD3=	.67	.49	.51	.72	.75	.61
		SE1=	.15	.11	.11	.16	.17	.14
18	20		1.55	2.15	2.15	2.60	2.80	2.95
		SD3=	.76	.37	.37	.68	.77	.69
		SE1=	.17	.08	.08	.15	.17	.15

	.45	1.55	1.75	1.95	2.15	2.35	2.65
SD3=	.69	.60	.44	.39	.37	.49	.67
SE1=	.15	.14	.10	.09	.08	.11	.15
	1.25	1.75	1.95	1.95	1.95	2.00	2.00
SD3=	.79	.55	.22	.22	.22	0.00	0.00
SE1=	.18	.12	.05	.05	.05	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	.40
SD3=	0.00	0.00	0.00	0.00	0.00	0.00	.50
SE1=	0.00	0.00	0.00	0.00	0.00	0.00	.11
	0.00	0.00	0.00	0.00	0.00	0.00	.35
SD3=	0.00	0.00	0.00	0.00	0.00	0.00	.59
SE1=	0.00	0.00	0.00	0.00	0.00	0.00	.13
	.10	1.15	1.90	1.95	2.00	2.00	2.00
SD3=	.31	.59	.31	.22	0.00	0.00	0.00
SE1=	.07	.13	.07	.05	0.00	0.00	0.00
	.10	.85	1.55	1.90	2.00	2.00	2.00
SD3=	.31	.37	.51	.31	0.00	0.00	0.00
SE1=	.07	.08	.11	.07	0.00	0.00	0.00
	.05	.85	1.00	1.10	1.20	1.25	1.55
SD3=	.22	.37	0.00	.31	.41	.44	.51
SE1=	.05	.08	0.00	.07	.09	.10	.11

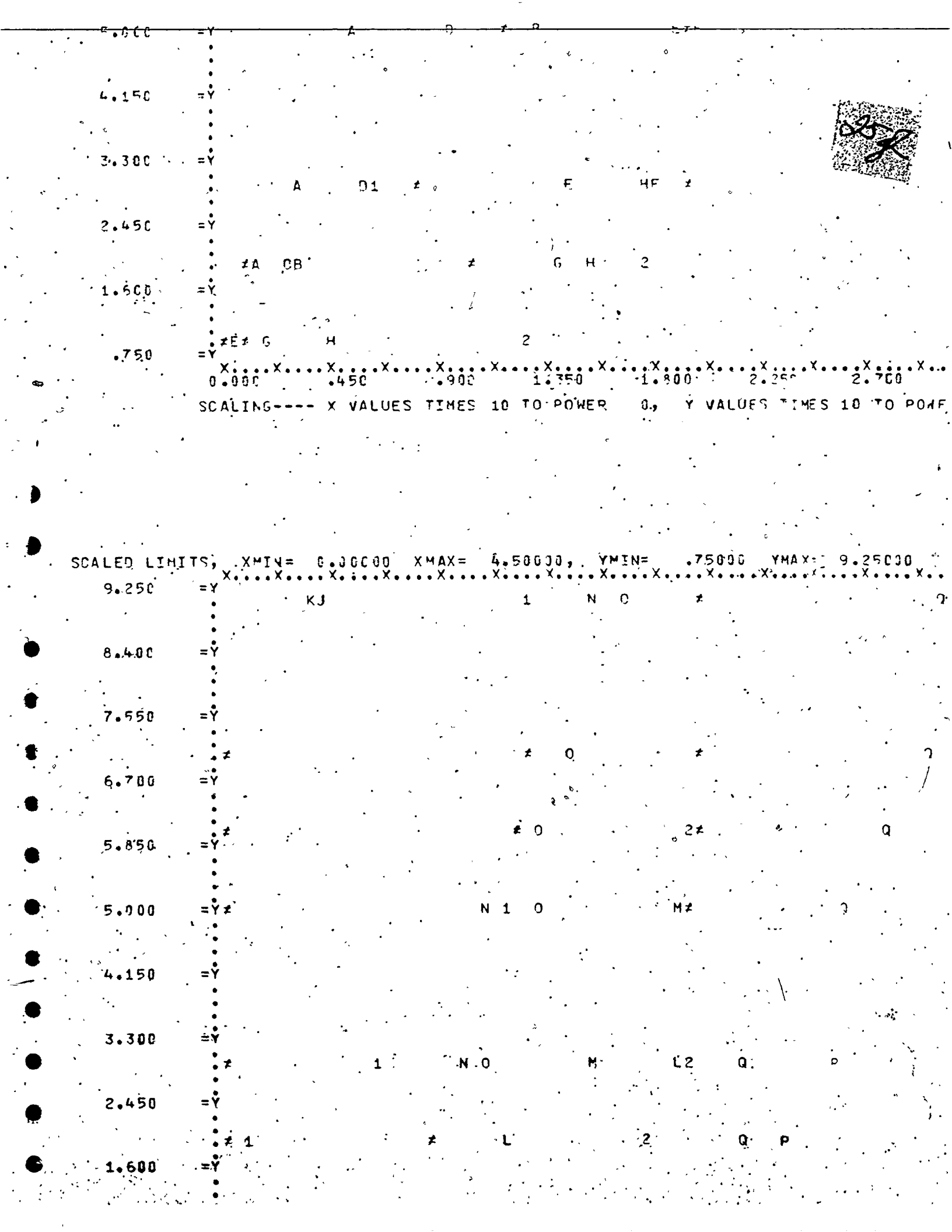
	.65	.85	1.10	1.30	1.30	1.45	1.65
SD3=	.22	.37	.31	.47	.47	.51	.49
SE1=	.05	.08	.07	.11	.11	.11	.11
	1.65	2.35	2.55	3.25	3.35	3.50	3.55
SD3=	.67	.49	.51	.72	.75	.61	.60
SE1=	.15	.11	.11	.16	.17	.14	.14
	1.95	2.15	2.15	2.60	2.80	2.95	3.00
SD3=	.76	.37	.37	.68	.77	.69	.65
SE1=	.17	.08	.08	.15	.17	.15	.15

```
                        .15           .11
  18      20                 1.55      2.15      2.15      2.60      2.8       2.95
                SD3=          .76       .37       .37       .68       .7?       .69
                SE1=          .17       .08       .08       .15       .1?       .15

SCALED LIMITS,  XMIN=  0.00000  XMAX=  4.50000,  YMIN=  .75000  YMAX=  9.25000
                 Y....X....X....X....X....X....X....X....X....X....X....X....X....X.
      9.250     =Y                        ≠   1   C    B      ≠   F          H
                 .
                 .
      8.400     =Y
                 .
                 .
      7.550     =Y
                 .
                 .                  A  D   C1    B        E2 F    H     G
      6.700     =Y
                 .
                 .            A       D   C1  B        E2F   H      G
      5.850     =Y
                 .
                 .
      5.000     =Y            A       D   ≠   B        E≠F   G   [redacted]
                 .
                 .
      4.150     =Y
                 .
```

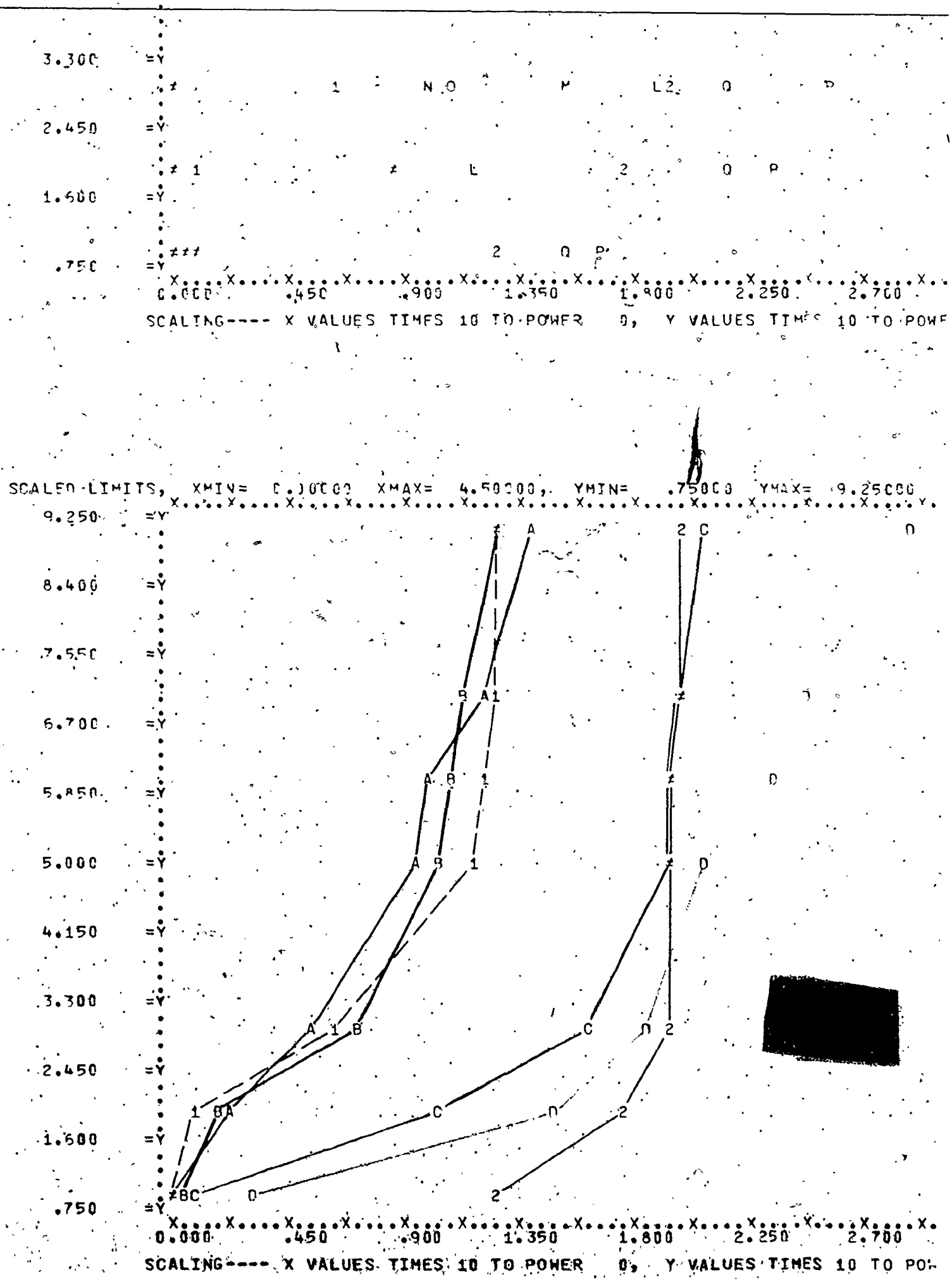
6	2.60	2.8	2.95	3.00
7	.68	.77	.69	.65
8	.15	.17	.15	.15

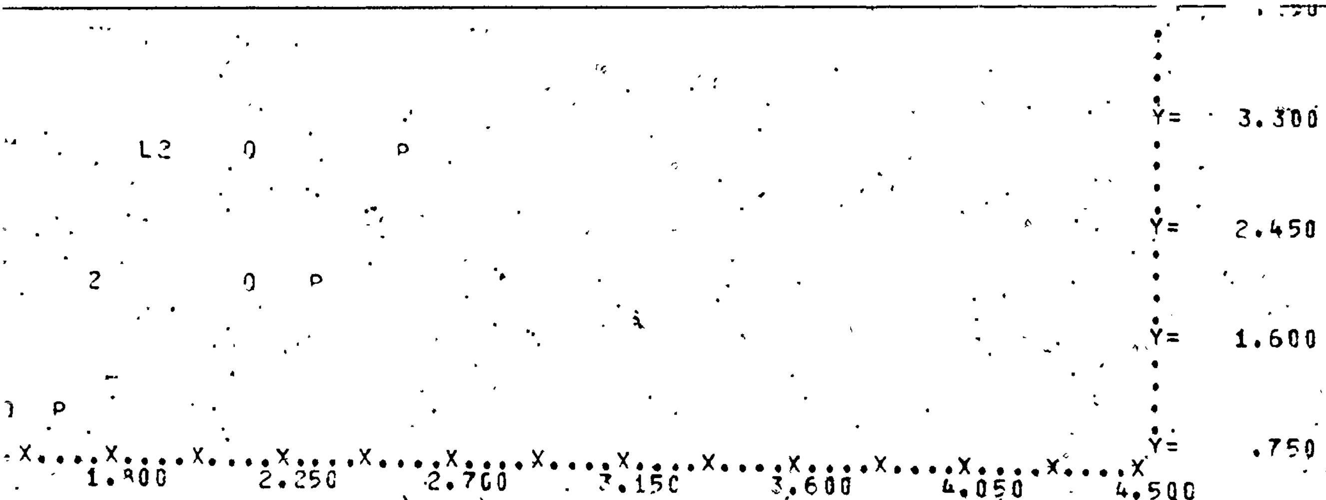
YMIN= .75000 YMAX= 9.25000 70 POINTS PLOTTED(≠ DENOTES COINCIDENT POINTS)

```
X....X....X....X....X....X....X....X....X....X....X....X....X....X....X
 B     ≠   F          H          G                                    Y=   9.250
                                                                      Y=   8.400
                                                                      Y=   7.550
    E2 F     H    G                                                   Y=   6.700
    E2F   H       G                                                  Y=   5.850
   E≠F   G                                                           Y=   5.000
                                                                      Y=   4.150
```


4.150
3.300
2.450
1.600
.750
0.000
.450
.900
1.350
1.800
2.250
2.700
SCALING---- X VALUES TIMES 10 TO POWER 0., Y VALUES TIMES 10 TO POWE
SCALED LIMITS, XMIN= 0.00000 XMAX= 4.50000, YMIN= .75000 YMAX= 9.25000
9.250
8.400
7.550
6.700
5.850
5.000
4.150
3.300
2.450
1.600

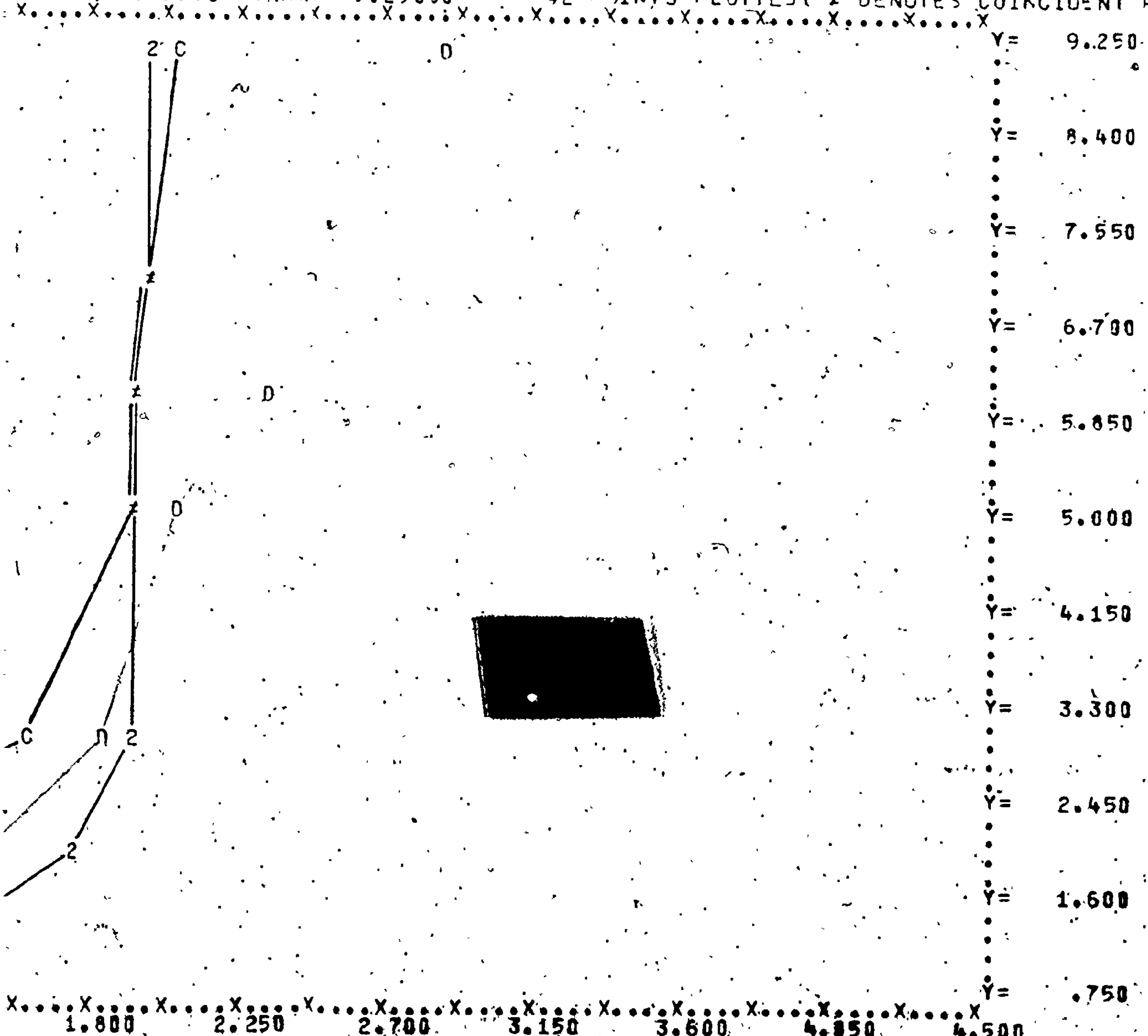
```
  .F2F  H.     G
                                                                    Y=    5.850

     EXF   G                                                        Y=    5.000

                                                                    Y=    4.150

                                                                    Y=    3.300
  HF   ≠
                                                                    Y=    2.450
  2
                                                                    Y=    1.600

                                                                    Y=     .750
X....X....X....X....X....X....X....X....X....X....X....X....X....X
   1.800      2.250      2.700      3.150      3.600      4.050      4.500
R   0,  Y VALUES TIMES 10 TO POWER   0


YMIN=    .75000   YMAX=  9.25000        70 POINTS PLOTTED( ≠ DENOTES COINCIDENT POINTS)
X....X....X....X....X....X....X....X....X....X....X....X....X....X Y=   9.250
 0       ≠                       Q           P
                                                                    Y=    8.400

                                                                    Y=    7.550
         ≠                    Q           P
                                                                    Y=    6.700
        2≠                Q           P
                                                                    Y=    5.850

       M≠             Q           P.          [redacted]            Y=    5.000

                                                                    Y=    4.150

                                                                    Y=    3.300
       L2   Q        P
                                                                    Y=    2.450
  2             P
```


3.300 =Y
2.450 =Y
1.600 =Y
.750 =Y
0.000 .450 .900 1.350 1.800 2.250 2.700
SCALING---- X VALUES TIMES 10 TO POWER 0, Y VALUES TIMES 10 TO POWE
SCALED LIMITS, XMIN= 0.00000 XMAX= 4.50000, YMIN= .75000 YMAX= 9.25000
9.250 =Y
8.400 =Y
7.550 =Y
6.700 =Y
5.850 =Y
5.000 =Y
4.150 =Y
3.300 =Y
2.450 =Y
1.600 =Y
.750 =Y
0.000 .450 .900 1.350 1.800 2.250 2.700
SCALING---- X VALUES TIMES 10 TO POWER 0, Y VALUES TIMES 10 TO PO

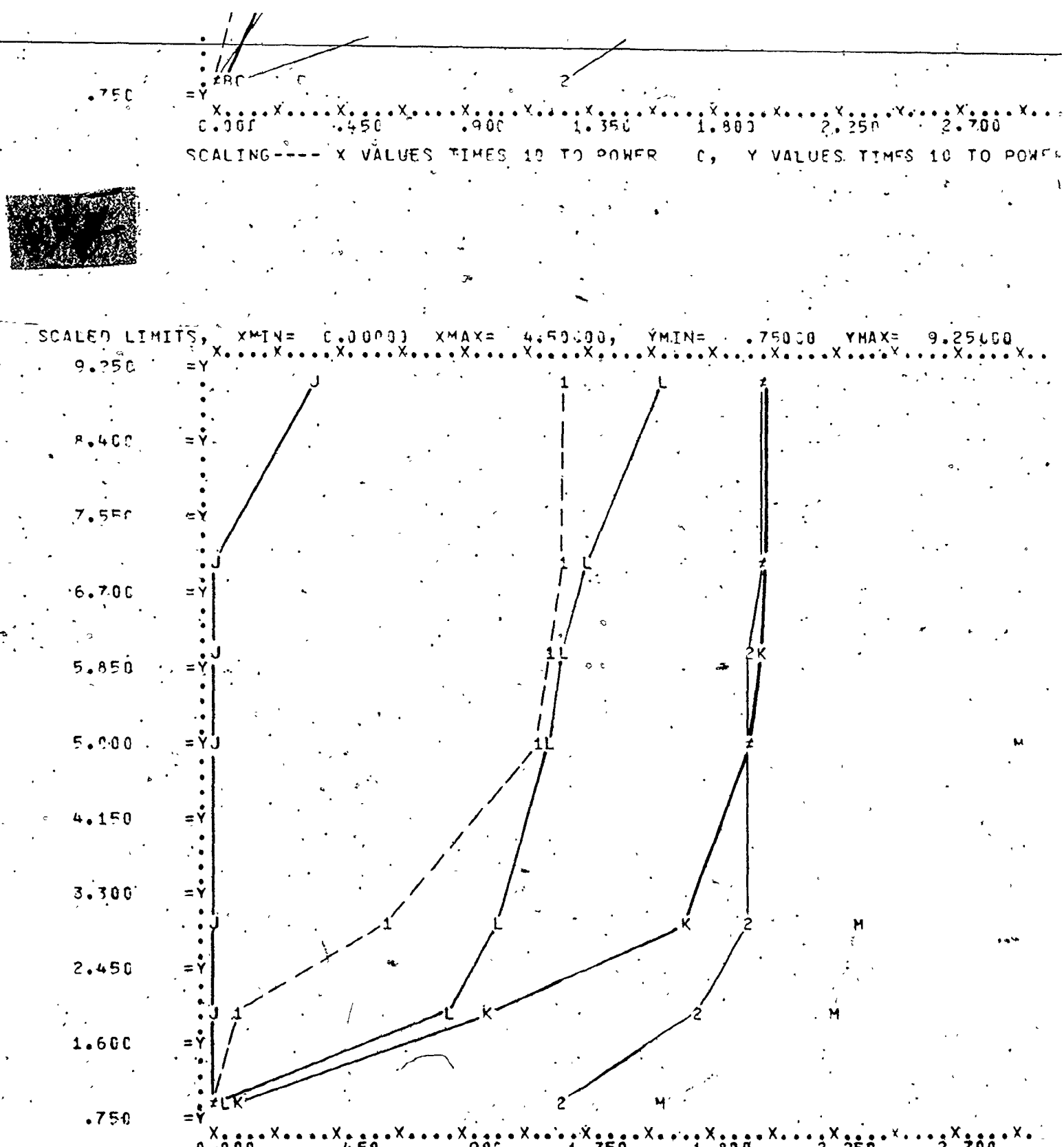


R 0, Y VALUES TIMES 10 TO POWER 0

YMIN= .75000 YMAX= 9.25000 42 POINTS PLOTTED(≠ DENOTES COINCIDENT POINTS)



R 0, 10



1	40	0.00	.23	.55	.93	.9[illegible]	1.20
2	40	.05	.18	.70	1.05	1.0[illegible]	1.13
3	40	.08	1.05	1.63	1.95	1.95	2.00
4	40	.33	1.48	1.85	2.05	2.33	2.48

```
                                                                    Y=     .750
...X....X....X....X....X....X....X....X....X....X....X....X....X....X....X
  1.350     1.800     2.250     2.700     3.150     3.600     4.050     4.500
0 POWER   C,  Y VALUES TIMES 10 TO POWER   0
```



```
TO POWER   0,  Y VALUES TIMES 10 TO POWER   0
     .55       .93       .98      1.20      1.38
     .70      1.05      1.09      1.13      1.28

    1.63      1.95      1.95      2.00      2.08
    1.85      2.05      2.33      2.48      2.90
    0.00      0.00      0.00      0.00       .38
```

3	40	.08	1.05	1.63	1.95	1.95	2.00
4	40	.33	1.48	1.85	2.05	2.33	2.48
5	40	0.00	0.00	0.00	0.00	0.00	0.00
6	40	.10	1.00	1.73	1.93	2.00	2.00
7	40	.05	.85	1.05	1.20	1.25	1.35
8	40	1.60	2.25	2.35	2.93	3.08	3.23

I= 1 NUM= 20 MEAN= 5.60 SD= 1.82 SE= .41
I= 2 NUM= 20 MEAN= 8.60 SD= 3.10 SE= .69
I= 3 NUM= 20 MEAN= 6.90 SD= 2.61 SE= .58
I= 4 NUM= 20 MEAN= 6.10 SD= 2.47 SE= .55
I= 5 NUM= 20 MEAN= 4.80 SD= .95 SE= .21
I= 6 NUM= 20 MEAN=10.30 SD= 1.45 SE= .33
I= 7 NUM= 20 MEAN=11.15 SD= 1.69 SE= .38
I= 8 NUM= 20 MEAN=13.95 SD= 2.21 SE= .49
I= 9 NUM= 20 MEAN=12.85 SD= 2.50 SE= .56
I= 10 NUM= 20 MEAN=12.85 SD= 1.46 SE= .33
I= 11 NUM= 20 MEAN= .40 SD= .50 SE= .11
I= 12 NUM= 20 MEAN= .35 SD= .59 SE= .13
I= 13 NUM= 20 MEAN=11.10 SD= .97 SE= .22
I= 14 NUM= 20 MEAN=10.40 SD= .94 SE= .21
I= 15 NUM= 20 MEAN= 7.00 SD= 1.49 SE= .33
I= 16 NUM= 20 MEAN= 7.70 SD= 1.98 SE= .44
I= 17 NUM= 20 MEAN=20.20 SD= 3.47 SE= .78
I= 18 NUM= 20 MEAN=17.20 SD= 3.21 SE= .72

UNSORTED DATA

I= 1 4.0 5.0 4.0 4.0 7.0 4.0 5.0 8.0 9.0 6.0 5.0 5

UNSORTED DATA

I= 2 10.0 10.0 5.0 9.0 6.0 4.0 5.0 2.0 2.0 2.0 2.0 1

UNSORTED DATA

I= 3 11.0 8.0 5.0 10.0 7.0 9.0 5.0 11.0 9.0 8.0 5.0 7

UNSORTED DATA

I= 4 5.0 4.0 4.0 4.0 6.0 4.0 4.0 7.0 10.0 5.0 5.0 5

UNSORTED DATA

I= 5 5.0 4.0 4.0 4.0 5.0 4.0 4.0 3.0 7.0 5.0 5.0 5

UNSORTED DATA

I= 6 11.0 11.0 10.0 10.0 11.0 9.0 11.0 12.0 12.0 10.0 8.0 11

UNSORTED DATA

I= 7 11.0 11.0 10.0 11.0 11.0 10.0 10.0 16.0 11.0 11.0 8.0 11

[illegible]	1.95	1.[illegible]	2.0[illegible]	2.[illegible]
[illegible]	2.05	2.[illegible]	2.4[illegible]	2.99
0	0.00	0.0[illegible]	0.0[illegible]	.38
3	1.93	2.00	2.00	2.00
[illegible]	1.20	1.2[illegible]	1.3[illegible]	1.60
[illegible]	2.93	3.0[illegible]	3.23	3.2[illegible]

.41
.69
.58
.55
.21
.33
.38
.49
.56
.33
.11
.13
.22
.21
.33
.44
.78
.72

5.0 8.0 9.0 6.0 5.0 5.0 4.0 4.0 8.0 5.0 5.0 5.0 10.0 5.0

5.0 2.0 2.0 2.0 2.0 1.0 4.0 0.0 1.0 2.0 0.0 4.0 2.0 1.0

5.0 11.0 9.0 9.0 5.0 7.0 11.0 5.0 4.0 4.0 5.0 4.0 5.0 4.0

4.0 7.0 10.0 5.0 5.0 5.0 6.0 6.0 5.0 7.0 9.0 6.0 14.0 6.0

4.0 3.0 7.0 5.0 5.0 5.0 6.0 6.0 6.0 5.0 4.0 4.0 5.0 5.0

11.0 12.0 12.0 10.0 8.0 11.0 12.0 6.0 10.0 10.0 10.0 10.0 10.0 12.0

10.0 16.0 11.0 11.0 8.0 11.0 15.0 12.0 11.0 11.0 11.0 10.0 11.0 11.0

DATA

I= 7 11.0 11.0 10.0 11.0 11.0 10.0 10.0 16.0 11.0 11.0 8.0 11.

UNSORTED DATA

I= 8 13.0 10.0 12.0 12.0 14.0 13.0 13.0 20.0 15.0 14.0 12.0 13.

UNSORTED DATA

I= 9 11.0 9.0 9.0 10.0 11.0 13.0 10.0 16.0 14.0 14.0 10.0 13.

UNSORTED DATA

I= 10 14.0 13.0 9.0 13.0 14.0 10.0 14.0 14.0 14.0 14.0 12.0 14.

UNSORTED DATA

I= 11 1.0 1.0 0.0 1.0 0.0 0.0 1.0 0.0 0.0 1.0 0.0 0.

UNSORTED DATA

I= 12 0.0 0.0 0.0 1.0 0.0 0.0 1.0 1.0 2.0 1.0 0.0 0.

UNSORTED DATA

I= 13 11.0 11.0 9.0 11.0 11.0 10.0 10.0 11.0 11.0 11.0 13.0 11.

UNSORTED DATA

I= 14 11.0 10.0 9.0 11.0 10.0 10.0 9.0 11.0 11.0 11.0 12.0 11

UNSORTED DATA

[redacted]

I= 15 7.0 7.0 6.0 6.0 10.0 6.0 5.0 9.0 9.0 5.0 6.0 6

UNSORTED DATA

I= 16 7.0 7.0 6.0 6.0 11.0 7.0 6.0 11.0 10.0 5.0 6.0 5

UNSORTED DATA

I= 17 19.0 15.0 13.0 18.0 20.0 23.0 20.0 24.0 23.0 16.0 18.0 18

UNSORTED DATA

I= 18 21.0 17.0 13.0 14.0 15.0 15.0 15.0 23.0 22.0 18.0 12.0 17

M= 1 STUDT1= .81 STUDTP= .56 STUDTQ= 2.70 STUDT2= .41 STUDT3= 1.99

TGN4= 210.0 TGN5= 1524.0 SD2= 3.29 STUDT4= .09 STUDT5= 2.11

M= 2 STUDT1= .49 STUDTP= .16 STUDTQ= 2.35 STUDT2= .39 STUDT3= 4.62

TGN4= 218.0 TGN5= 1338.0 SD2= 1.96 STUDT4= .06 STUDT5= 3.03

M= 3 STUDT1= .38 STUDTP= 2.02 STUDTQ= 1.24 STUDT2= 2.23 STUDT3= .75

TGN4= 429.0 TGN5= 4703.0 SD2= 1.62 STUDT4= 2.11 STUDT5= .98

10.0 16.0 11.0 11.0 8.0 11.0 15.0 12.0 11.0 11.0 11.0 10.0 11.0 11.0

13.0 20.0 15.0 14.0 12.0 13.0 14.0 13.0 15.0 13.0 14.0 16.0 15.0 18.0

10.0 16.0 14.0 14.0 10.0 13.0 14.0 13.0 18.0 15.0 16.0 14.0 13.0 14.0

14.0 14.0 14.0 14.0 12.0 14.0 14.0 14.0 13.0 12.0 11.0 12.0 13.0 13.0

1.0 0.0 0.0 1.0 0.0 0.0 1.0 1.0 0.0 0.0 1.0 0.0 0.0 0.0

1.0 1.0 2.0 1.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0

10.0 11.0 11.0 11.0 13.0 11.0 13.0 12.0 12.0 12.0 11.0 11.0 11.0 10.0

9.0 11.0 11.0 11.0 12.0 11.0 12.0 11.0 18.0 10.0 10.0 11.0 9.0 9.0

5.0 9.0 9.0 5.0 6.0 6.0 7.0 6.0 6.0 7.0 7.0 8.0 10.0 7.0

6.0 11.0 10.0 5.0 6.0 5.0 8.0 6.0 7.0 8.0 10.0 8.0 10.0 10.0

20.0 24.0 23.0 16.0 18.0 18.0 16.0 24.0 24.0 24.0 23.0 22.0 20.0 24.0

15.0 23.0 22.0 18.0 12.0 17.0 14.0 16.0 22.0 16.0 20.0 20.0 16.0 18.0

.70 STUDT2= .41 STUDT3= 1.99 NUM2= 40 AMEAN2= 5.25

14= .09 STUDT5= 2.11

35 STUDT2= .39 STUDT3= 4.62 NUM2= 40 AMEAN2= 5.45

14= .06 STUDT5= 3.03

24 STUDT2= 2.23 STUDT3= .76 NUM2= 40 AMEAN2= 18.73

4= T5= .98

... 218.0 ... 1338.0 ... 1.95 ... .06 ...

M= 3 STUDT1= .38 STUDTP= 2.02 STUDTQ= 1.24 STUDT2= 2.23 STUDT3= .75

TGN4= 429.0 TGN5= 4703.0 SD2= 1.62 STUDT4= 2.11 STUDT5= .98

M= 4 STUDT1= .37 STUDTP= 2.92 STUDTQ= .41 STUDT2= 2.35 STUDT3= 0.00

TGN4= 536.0 TGN5= 7406.0 SD2= 2.39 STUDT4= 2.59 STUDT5= .20

M= 5 STUDT1= .06 STUDTP= 2.76 STUDTQ= 8.06 STUDT2= 2.75 STUDT3= 7.94

TGN4= 15.0 TGN5= 17.0 SD2= .54 STUDT4= 2.75 STUDT5= 8.01

M= 6 STUDT1= .52 STUDTP= 2.67 STUDTQ= 1.00 STUDT2= 2.35 STUDT3= 1.41

TGN4= 430.0 TGN5= 4662.0 SD2= 1.01 STUDT4= 2.48 STUDT5= 1.18

M= 7 STUDT1= .28 STUDTP= .60 STUDTQ= 2.81 STUDT2= .78 STUDT3= 2.10

TGN4= 294.0 TGN5= 2282.0 SD2= 1.75 STUDT4= .69 STUDT5= 2.40

M= 8 STUDT1= .63 STUDTP= 3.72 STUDTQ= 1.95 STUDT2= 3.15 STUDT3= 1.23

TGN4= 748.0 TGN5= 14502.0 SD2= 3.63 STUDT4= 3.23 STUDT5= 1.40

JDT4= .06 STUDT5= 3.07

1.24 STUDT2= 2.23 STUDT3= .75 NUM2= 40 AMEAN2= 10.73

JDT4= 2.11 STUDT5= .98

.41 STUDT2= 2.35 STUDT3= 0.00 NUM2= 40 AMEAN2= 13.40

JDT4= 2.59 STUDT5= .20

8.06 STUDT2= 2.75 STUDT3= 7.94 NUM2= 40 AMEAN2= .38

JDT4= 2.75 STUDT5= 8.01

1.00 STUDT2= 2.35 STUDT3= 1.41 NUM2= 40 AMEAN2= 10.75

UDT4= 2.48 STUDT5= 1.18

2.81 STUDT2= .78 STUDT3= 2.10 NUM2= 40 AMEAN2= 7.35

UDT4= .69 STUDT5= 2.40

1.95 STUDT2= 3.15 STUDT3= 1.23 NUM2= 40 AMEAN2= 18.70

UDT4= 3.23 STUDT5= 1.40

```
        MCMASTER-SCOPE 3.4.3   397      06/30/76
14.05.01.HCBB04J   FROM
14.05.01.IP   00001728 WORDS - FILE INPUT , DC CC
14.05.01.HCBB,T40.
14.05.01.                                BENTLEY,M.
14.05.01.ROUTE,OUTPUT,DEF,FC=U2.
14.05.19.FTN.
14.05.42./ 440008   CM STORAGE USED
14.05.43./T    2.433 CP SECONDS COMPILATION TIME
14.05.43.LGO.
14.07.23.     STOP
14.07.23.      2.172 CP SECONDS EXECUTION TIME
14.07.23.OP   00008000 WORDS - FILE OUTPUT , DC 40
14.07.24.MS    10752  WORDS  (     7168 MAX USED)
14.07.24.CPA       8.967 SEC.          4.035 ADJ.
14.07.24.IO        4.015 SEC.           .401 ADJ.
14.07.24.CM      226.642 KWS.          2.371 ADJ.
14.07.24.SS                            6.808
14.07.24.PP        7.559 SEC.      DATE 09/19/76
14.07.24.COMPUTER SECONDS USED                    17.056
14.07.24.COST (AT $ 378/COMPUTER HOUR)            $1.79
14.07.24.EJ  END OF JOB, **

**********          HCBB04J  //// END OF LIST ////
**********          HCBB04J  //// END OF LIST ////
```

[redacted]