A BIOMETRICAL-GENETICAL STUDY OF THE EFFECTS AND INTERACTIONS OF THE PARASITE, THE HOST AND THE ENVIRONMENT ON GROWTH RATE AND AGGRESSIVENESS OF USTILAGO HORDEL (PERS.) LAGERH

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A Biometrical-Genetical Study of the Effects and Interactions of the Parasite, the Host and the Environment on Growth Rate and Aggressiveness of *Ustilago hordei* (Pers.) Lagerh.

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ABSTRACT

From two teliospores of *Ustilago hordei* (Pers.) Lagerh. (the fungus causing the covered smut disease of barley) the eight monokaryons and the sixteen possible dikaryons derived from the monokaryotic matings, were used in a biometrical genetic study. Evaluations were made of the relative roles of the genotype, environment and genotype-environment interactions in determining growth rate of the monokaryons and aggressiveness of the dikaryons (parasites). The genetics of host-parasite relationships was also examined using different barley cultivars as hosts.

The genotype, the environment, and the interaction of the two were found to contribute significantly to the control of variability in growth rate of the monokaryons. Variability in degree of infection of the dikaryons was controlled by the environment, the genotype of the parasite (aggressiveness), and the genotype of the host (tolerance). There were no significant effects from parasite-environment interactions or from host-parasite interactions.

The aggressiveness of the dikaryons (parasites) and the growth rates of the parental monokaryons were positively correlated, suggesting that both characters may be under the control of a common gene system. It was also demonstrated that faster growth rate in the monokaryons was accompanied by an increased sensitivity to environmental change.

A general biometrical-genetical model was developed to describe the relationships and interactions within a host-parasite-environment system.

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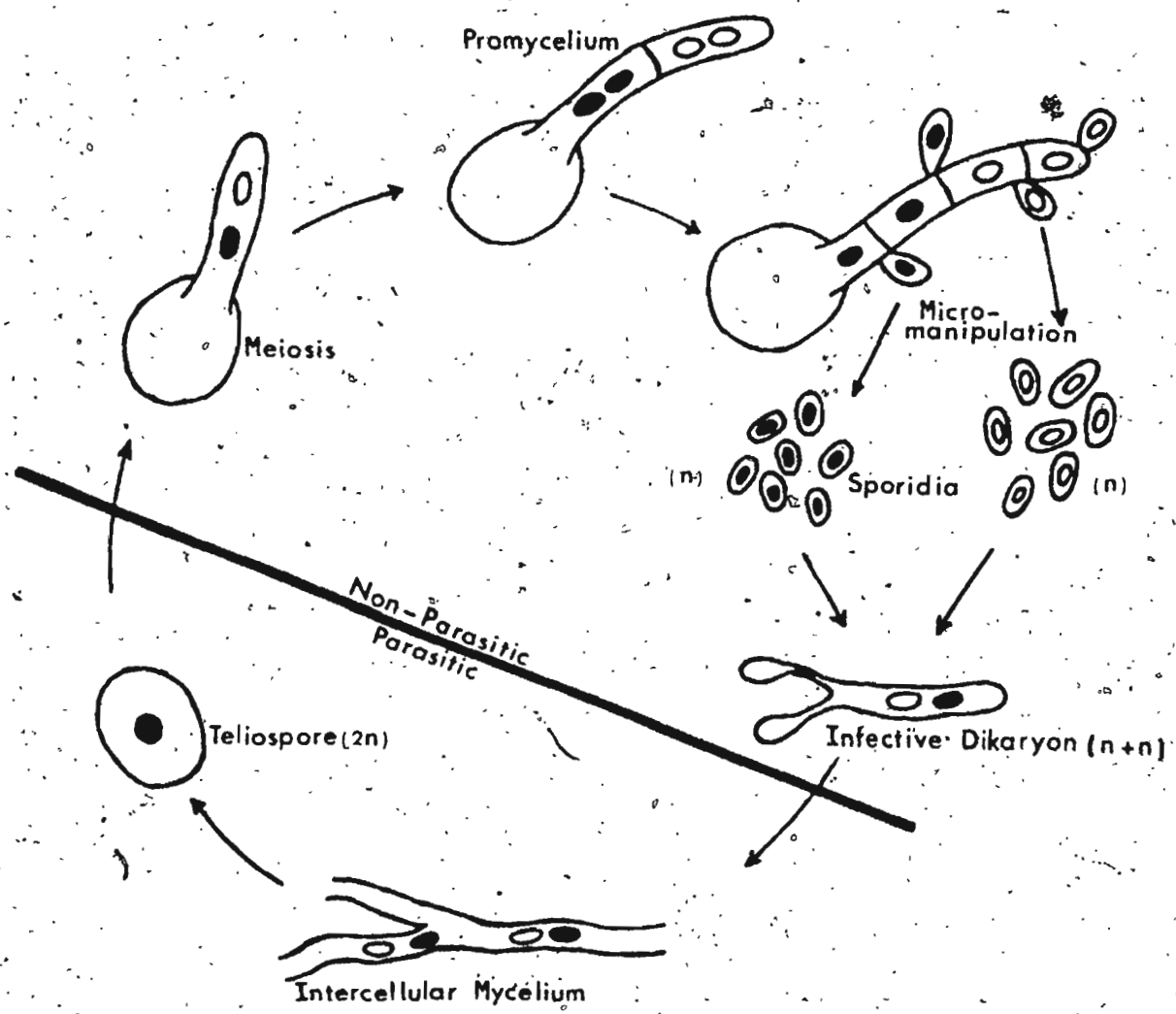
INTRODUCTION

The causal organism in the covered smut disease of cultivated barley *Hordeum vulgare* L. is the fungus *Ustilago hordei* (Pers.) Lagerh., a member of the Basidiomycetes. *U. hordei* is well suited for genetic studies as its simple life cycle (Fig. 1) permits easy culturing and mating of the haploid cells. Meiosis occurs *in vitro* during germination of the diploid teliospore which forms an ordered tetrad, a four-celled promycelium. Each cell, a haploid gamete, reproduces by budding to form sporidia (monokaryons). Using micromanipulation these monokaryons can be removed and established in four separate cultures as vegetative clones. The mating system is di-polar and the mating type of each of the four cultures can be determined by the Bauch test (Bauch, 1927). Two of them will be of mating type (+) and the other two (-). Once this has been accomplished, controlled matings of the compatible monokaryons will produce the obligately parasitic, dikaryotic phase of the fungus to be used in the inoculation of barley seeds.

In parasitic systems, there are two main aspects of host-parasite relationships. First, whether or not infection is possible. If it is, the parasite is virulent and the host susceptible; if not, the parasite is avirulent and the host resistant. In the case of covered smut, like other fungal diseases, the possibility of infection, in a particular environment, depends on specific relationships between the barley cultivar (host genotype) and the physiological race (parasite genotype) of *U. hordei* (Tapke, 1945). Evidence indicates this aspect of the

Fig. 1. Life cycle of *Ustilago hordei*. (After Hood, 1966)


Promycelium
Meiosis
Micro-
manipulation
(n)
Sporidia
(n)
Non-Parasitic
Parasitic
Teliospore (2n)
Infective Dikaryon (n+n)
Intercellular Mycelium

specific relationship is controlled by a small number of major genes (Sidhu & Person, 1971) with the existence of gene-for-gene relationships (Sidhu & Person, 1972). The second aspect of host-parasite relationships is the degree of infection (when the parasite is virulent and the host susceptible) which depends on the aggressiveness of the parasite and tolerance of the host (Van der Plank, 1968; Emara, 1972). Emara (1972) showed that aggressiveness in *U. hordei* is a continuous (quantitative) character controlled by polygenes.

Quantitative genetic studies often become too involved for practical purposes when the character under investigation is assessed in more than one environment, because of alterations in genetic expression which frequently occur, with changing environmental conditions. These alterations are definable as genotype-environment interactions and can be an important source of phenotypic variation. A similar type of relationship may occur in host-parasite relationships, between two genetic systems, that is host-parasite interaction.

In the present investigation, the variations in monokaryotic growth rate and dikaryotic infectivity (aggressiveness of the parasite genotypes) were examined in terms of their genetic, environmental, and genotype--environment interaction components to determine the relative importance of each and for comparative estimates of the stability of expression of the genotypes used in this study. Parasitic aggressiveness was also examined with respect to certain aspects of parasitism, specifically those aspects which deal with variation in degree of infection due to differences in aggressiveness among the parasites, differences in tolerance

among the hosts, and possible interactions between parasites and hosts.

U. hordei, being an obligate parasite, has the obvious disadvantage of requiring the tissue of the host plant to carry it through the infective dikaryon stage. The disease is undetectable until the barley plant matures, which may require a waiting period of several months if environmental conditions are not totally favorable for the growth of the plant. For this reason, various monokaryon phenotypic characters were examined in the hope that some insight into the relative aggressiveness of the parasites might be obtained from studying phenotypic characteristics of the parental monokaryons in culture.

Information gained from this study on aggressivess and growth of *U. hordei* could help a great deal in studying and understanding tolerance in barley which in turn would aid in developing highly tolerant cultivars, in other words cultivars with long lasting horizontal resistance (Van der Plank, 1968). Moreover, this information may indicate methods of studying other fungi with the aim of developing highly aggressive strains, to be used in the biological control of insect pests and vectors. (Huffaker, 1971).

LITERATURE REVIEW

Jones (1924) discusses the importance of the environment in plant disease occurence, and demonstrates cases in which the amount of growth of the pathogen, where this is measured in response to environmental changes, nearly parallels the amount of disease caused by the same pathogen in response to the same environmental changes.

Reed and Faris (1924) demonstrate the interdependence of certain environmental factors and the importance of their interaction in determining the occurence of plant disease and the severity of infection.

Faris (1924) concludes that variation in percentage of infection by *Ustilago hordei* was not due solely to environmental factors but was also dependent upon the genetic constitution of the fungus itself.

The relationship between the pathogen and the infected plant is examined by Mode (1958) who makes particular reference to cereals and the rust fungi. Blake (1969) describes some general processes in plants which determine susceptibility or resistance to disease. Person (1967) and Van der Plank (1968) consider the genetic involvement in parasitism and discuss the relative roles and interactions of the genotypes in controlling virulence and resistance in parasite-host systems.
Van der Plank, (1968) also states that in the parasite, virulence seems often to be controlled by a small number of major genes, while aggressiveness is often controlled by polygenes. Emara (1972) found that aggressiveness in *U. hordei* is under the control of polygenes, and that the environment also is of great importance in the control of this character.

Mather and Jones (1958 a) explain how interaction of genotypes and environments can affect phenotypic expression and constitute an added source of variation. The simplest case they offer for consideration is that of two genotypes in two environments. The variation producing four phenotypes is determined by the genetic difference (d_a) between the two genotypes, the difference (e_1) in environmental effect between the two environments, and the statistical interaction (g_1) of the genetic and environment components (Table 1).

Table 1

The four phenotypes of two genotypes in two environments expressed in terms of parameters d_a, e_1, & g_1, representing genetic, environmental, and interactive effects.

	Genotype		
	AA	aa	Mean
Environments X	$d_a + e_1 + g_1$	$-d_a + e_1 - g_1$	e_1
Y	$d_a - e_1 - g_1$	$-d_a - e_1 + g_1$	$-e_1$
Mean	d_a	$-d_a$	0

(From Mather & Jones, 1958 a).

The genotype-environment interaction parameter g_1 is described as the amount of variation ".....added by the interaction to the phenotypes of AA in environment X and aa in environment Y, and deducted from the phenotypes of AA in Y and aa in X".

Bucio Alanis (1966) and Bucio Alanis and Hill (1966), examine environmental and genotype-environment interaction components of

phenotypic variation in a practical analysis of data from an experiment on two inbred lines of *Nicotiana rustica* grown in sixteen environments and demonstrate a relationship between these components such that interaction increased with environmental effect.

Perkins & Jinks (1968) extend the analysis of Bucio Alanis (1966), and Bucio Alanis and Hill (1966) to cover any number of inbred lines and environments, and to include the case of unequal gene frequencies.

Pederson (1968), Westerman (1970 a,b,c) and Westerman and Lawrence (1970), examine the relative roles of the genotype and environment, and their interactions in the self-fertilizing plant *Arabidopsis thaliana*, and conclude that genotype-environment interactions contribute significantly to variations in various developmental processes of the plant.

The significance of genotype-environment interactions in grasses is discussed by Breese (1969) and Wright (1971), in wheat by Baker (1969) and Easton & Clements (1973), in barley by Paroda & Hayes (1971) and in cultivated rape by Whitcombe and Whittington (1971). It is quite clear that for investigations of variability, reliable estimates of genotype-environment interactions should be of primary consideration.

Fripp (1972) and Fripp & Caten (1971, 1973) emphasize the importance of genotype-environment interactions in growth rate of the fungus *Schizophyllum commune*.

To date, very few biometrical-genetical studies have been made on the host tolerance or the parasite aggressiveness (Emara, 1972; Emara & Sidhu, 1974). Moreover, no attempt has been made to study the host, parasite and environment together with their interactions. In the present investigation

such a study was carried out on the *U. hordei*-barley system using newly developed techniques and models in biometrical genetics.

MATERIALS & METHODS

1. Biological Material

The eight monokaryons (Table 2) used in this study were derived from two teliospores designated E and F, originally collected from the Canadian prairies. All these monokaryons carry the recessive virulence allele *Uh v-1* (Sidhu and Person, 1971). The five highly inbred cultivars of barley used as hosts are listed in Table 5.

2. Medium

Preceding each experiment, cultures of the eight monokaryons were grown in Vogel's (1956) complete liquid medium prepared as follows:

Difco yeast extract	5 g.
salt-free casein hydrolysate (N.B. Co.)	5 g.
dextrose	10 g.
tryptophane	50 mg.
Vogel's salt solution	20 ml.
vitamin solution (Holliday, 1961)	10 ml.
distilled water	1 litre

For solid medium, 2% Difco bacto agar was added.

The minimal medium used, contained 20 ml. Vogel's salt solution and 10 g. dextrose per litre of distilled water.

3. Propagation

An inoculum of each of the eight monokaryon cultures was placed in a 125 ml. DeLong culture flask containing 30 ml. Vogel's complete liquid medium and fitted with stainless steel closures. The flasks were placed on a New Brunswick culture shaker and the cultures allowed to grow for four days at room temperature. This procedure was carried

Table 2

The eight monokaryon genotypes

Monokaryons	Genotype Numbers
E_1	1
E_2	2
E_3	3
E_4	4
F_1	5
F_2	6
F_3	7
F_4	8

out before the beginning of each experiment.

4. Experimental.

(a). Growth Rate

The growth rate experiments were conducted in a Sherer controlled environment incubator. The fifteen environments used were produced by modifying either the incubator temperature or the concentrations of ingredients in the medium (Table 3). Fourteen of the fifteen environments were grouped into sets according to the proximity of physical conditions which constituted the differences between them (i.e. the temperature environments form set 1, the yeast environments form set 2, and so on). Since environment number 1 is composed of complete medium at the optimum temperature of 22°C, it is referred to as the standard environment and is included in each set for analysis and tabulation of growth measurements. The reason for the inclusion of the standard environment is that it represents one more level in the modification of the particular environmental condition of each set. That is to say, since it contains complete medium it has dextrose, casein and yeast extract concentrations of 1.0%, 0.5% and 0.5% respectively. For set 1 it represents the additional temperature level of 22°C. Environment number 15 was used to effect conditions which were highly adverse to the growth of the monokaryons.

For each environment, the monokaryons were prepared by removing 1 ml. of each liquid culture and diluting to 1:100,000 in sterile distilled water. 0.1 ml. aliquots of each diluted culture were spread on the appropriate agar medium in standard Petri plates and placed in the

Table 3

The composition of the fifteen environments for growth rate

	Environments	Environment Numbers
Set 1	Complete medium at 22°C	1
	Complete medium at 15°C	2
	Complete medium at 20°C	3
	Complete medium at 25°C	4
	Complete medium at 27.5°C	5
Set 2	1.0% dextrose at 22°C (complete medium)	1
	0.1% dextrose at 22°C	6
	0.5% dextrose at 22°C	7
	1.5% dextrose at 22°C	8
Set 3	0.50% casein at 22°C (complete medium)	1
	0.05% casein at 22°C	9
	0.25% casein at 22°C	10
	1.00% casein at 22°C	11
Set 4	0.50% yeast at 22° (complete medium)	1
	0.05% yeast at 22°C	12
	0.25% yeast at 22°C	13
	1.00% yeast at 22°C	14
	Minimal medium at 22°C	15

incubator for 96 hours.

The plates were arranged in four randomized complete blocks with each monokaryon replicated in two plates randomly distributed within each block.

At the end of 96 hours, the plates were removed from the incubator. Using a microscope fitted with an ocular micrometer calibrated in millimeters, the diameters of the colonies were measured as an index of growth rate. Five colonies per plate were chosen at random. The mean value for each plate constituted the basic observation in the analysis of variance.

(b). Degree of Infection

Approximately 150 seeds of the barley cultivar Hannchen were placed in each of sixteen seven-dram vials. The dikaryons (referred to throughout as parasites) were produced by combining 5 ml. of each of the two appropriate monokaryon liquid cultures (Table 4) in the vial labelled with the proper parasite number. Infection was achieved by allowing the seeds to remain in the inoculum for twenty minutes under vacuum. The excess inoculum was poured off and the seeds placed in small coin envelopes which were left open for three days to allow the seeds to air-dry.

The inoculated seeds were sown in the greenhouse in six inch pots at a depth of about one-quarter inch. Three seeds were placed in each pot since pre-testing of the stock seeds showed them to be of low germinability. After the seedlings appeared, the plants were thinned to one per pot.

Two randomized complete blocks were used, with each dikaryon

Table 4

The sixteen dikaryons (parasites) produced by all possible combinations of the eight monokaryons.

Parasite genotype numbers are shown in parentheses

	monokaryon numbers	E_2^+ 2	E_4^+ 4	$F_{2_4}^+$ 6	F_3^+ 7
E_1^-	1	E_1E_2 (1)	E_1E_4 (2)	E_1F_2 (3)	E_1F_3 (4)
E_3^-	3	E_3E_2 (5)	E_3E_4 (6)	E_3F_2 (7)	E_3F_3 (8)
F_1^-	5	E_2F_1 (9)	E_4F_1 (10)	F_1F_2 (11)	F_1F_3 (12)
F_4^-	8	E_2F_4 (13)	E_4F_4 (14)	F_2F_4 (15)	F_3F_4 (16)

represented in two rows of five plants each, randomly distributed within each block. A sixteen hour daylength was used during the course of the experiment. Degree of infection was recorded at maturity as percentage of infected heads per row. The mean of each two rows representing the same parasite in each block constituted the basic observations in the analyses. The data were transformed to arcsin to accommodate analysis of variance.

This procedure was carried out twice: in November 1972 and in October 1973. A similar experiment was conducted in the field in June 1973, using the barley cultivars Hannchen, Vantage, Lion, Trebi, and Odessa, as different hosts (Table 5). Inoculum preparation and seed infection was performed as before with the exception that two vials were used (one for each block for each parasite on each cultivar). In addition, each monokaryon was cultured in two flasks, each having 60 ml. of liquid medium, to facilitate infection on the additional four varieties of barley and increased number of seeds per cultivar. The infected seeds were sown in two randomized complete blocks. Each parasite was represented in five, 20-foot rows of plants randomly distributed within each block; each row consisting of 150 seeds of one of the five cultivars. Degree of infection was recorded at maturity in September 1973, as percentage of infected heads per row and transformed to arcsin to permit analysis of variance. Based on location and date of sowing, three environments are represented in the preceding experiments and will be referred to henceforth as the macro-environments (Table 6).

Table 5

The five barley cultivars and their numbers as host genotypes

Barley Cultivars	Host Genotypes
Hannchen (C.I. 541)	1
Vantage (C.I. 7324)	2
Lion (C.I. 923)	3
Trebi (C.I. 936)	4
Odessa (C.I. 182)	5

Table 6

The three macro-environments for degree of infection

Macro-environments	Macro-environment numbers
Greenhouse November, 1972	1
Greenhouse October, 1973	2
Field June, 1973	3

5. Analytical

Fixed model analyses of variance for factorial design were used to determine, primarily, the existence of differences among monokaryon genotypes, parasite genotypes, environments and host genotypes, and to test for the presence of interactions, the most important of which are those between the genotypes of the (+) and (-) monokaryons, between the monokaryons and environments, and parasites and environments and between parasites and hosts. Since the monokaryons are haploid cells they may be regarded as inbred lines. Thus, for estimates of the genetic effects, environmental effects, and genotype-environment interaction effects, the basic biometrical-genetical model for the general case of unequal gene frequencies among the genotypes was used (Perkins & Jinks, 1968; Perkins 1970).

$$P_{ij} = \hat{m} + \hat{d}_i + \hat{e}_j + \hat{g}_{ij}$$

where:

P_{ij} = performance of the ith genotype in the jth environment.

$\hat{m}$ = grand mean over all genotypes and environments

$\hat{d}_i$ = additive genetic effect of the ith genotype where $i = 1.....t$

$\hat{e}_j$ = additive environmental effect of the jth environment, where $j = 1.....s$

$\hat{g}_{ij}$ = effect of the interaction of the ith genotype and the jth environment.

The various components of the model may be estimated as follows:

$$\hat{m} = P../st$$

$$\hat{d}_i = P_{i.}/s - \hat{m}$$

$$\hat{e}_j = P_{.j}/t - \hat{m}$$

$$\hat{g}_{ij} = P_{ij} - \hat{m} - \hat{d}_i - \hat{e}_j$$

The term "performance" commonly used in the above model is synonymous with "phenotype", which in the present study will be referred to as either growth rate or degree of infection. The genotype-environment interactions ($\hat{g}_{ij}$'s) for each genotype were regressed on the environmental effects ($\hat{e}_j$'s) and the regression coefficients (b's) become a measure of the linear sensitivity to environmental change for each genotype, and the deviation mean squares (DMS's) are measures of the non-linear sensitivity to environmental change for each genotype(Fripp, Y.J. & Caten, C.E. 1973). Correlation coefficients (r's) were computed amongst the various aspects of the phenotype and the coefficients of determination (r^2's) gave a measure of the percentage of genes in common between any two characters.

RESULTS

1. Growth Rate

(a). The genetic and environmental effects, and their interactions.

The continuously variable nature of the rate of growth shown by the eight monokaryon genotypes is illustrated in Fig. 2. Such a continuous character is indicative of polygenic control and heritable variation, and hence readily lends itself to biometrical genetic studies.

The mean growth measurements for the eight monokaryon genotypes in each of the fifteen environments are given in Table 7 and represent a summary of the data obtained from the fifteen growth rate experiments. Analysis of variance for these data is presented in Table 8. The highly significant Environments M.S. confirms the effectiveness of the medium and temperature modifications in producing variability in growth rate. Also, the differences among monokaryon genotypes are shown to be highly significant. Of the three significant interactions, the genotype--environment interaction alone is of relevant importance. Table 9 shows the estimates of the genetic effect, $\hat{d}_i$, for each of the monokaryon genotypes, the environmental effect, $\hat{e}_j$, of each environment, and the 120 genotype-environment interaction effects, $\hat{g}_{ij}$'s, produced by the eight genotypes in the fifteen environments. The coefficients, b_i's, for the regression of $\hat{g}_{ij}$ on $\hat{e}_j$ for each

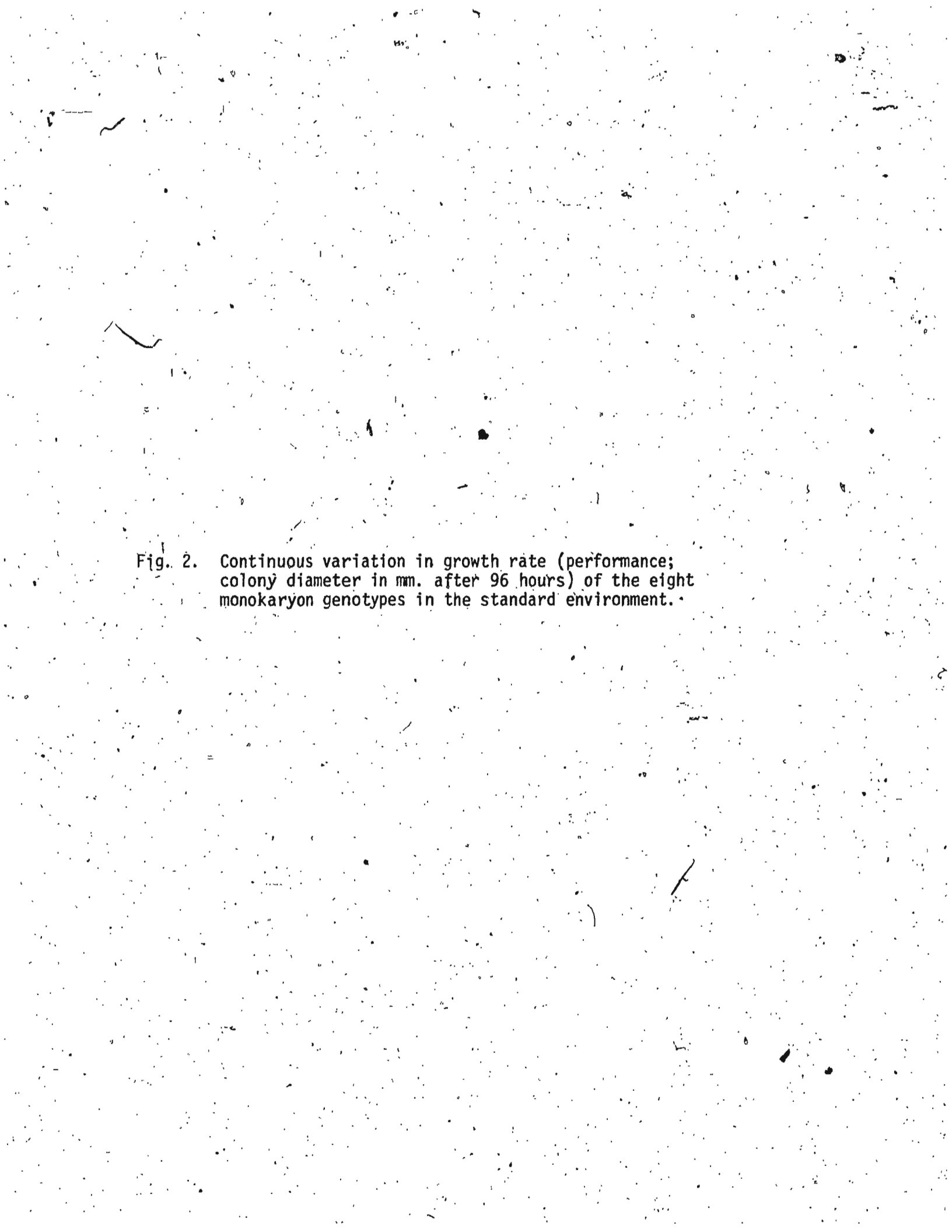


Fig. 2. Continuous variation in growth rate (performance; colony diameter in mm. after 96 hours) of the eight monokaryon genotypes in the standard environment.

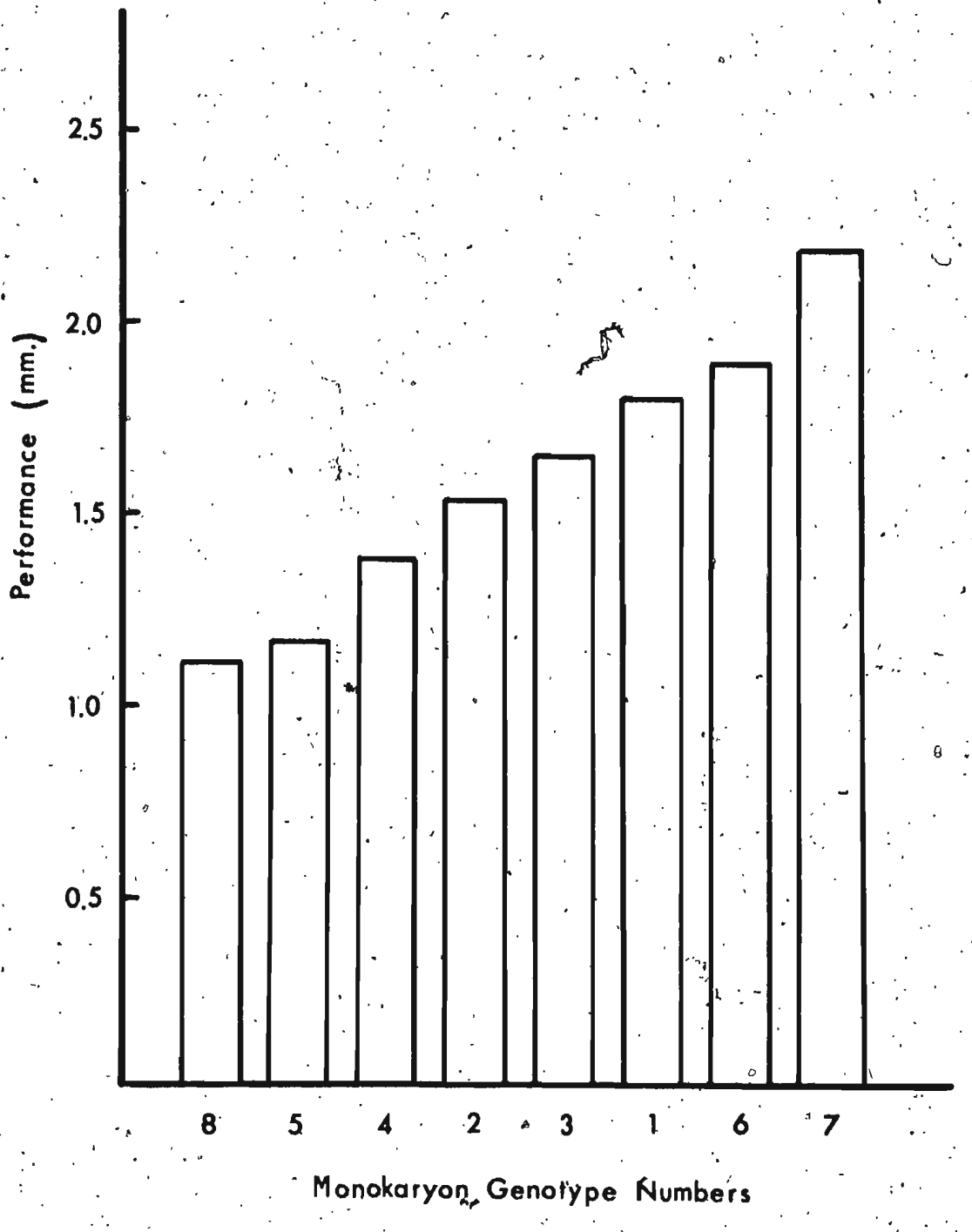

2.5
2.0
1.5
1.0
0.5
Performance (mm.)
8
5
4
2
3
1
6
7
Monokaryon Genotype Numbers

Table 7

Growth rates (mean colony diameters in millimeters) of the eight monokaryon genotypes across all fifteen environments.

Monokaryon Genotype Numbers	Environment Numbers															Me
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	1.79	0.33	1.30	1.43	0.98	1.45	1.33	0.98	1.51	1.60	0.69	0.53	0.70	0.72	0.26	1.1
2	1.52	0.28	1.34	1.26	0.58	0.84	1.11	1.31	2.14	1.04	1.43	1.27	1.66	0.98	0.55	1.1
3	1.65	0.25	1.14	1.01	0.78	1.34	1.38	1.06	1.03	1.02	0.90	0.77	0.87	1.16	0.34	0.1
4	1.38	0.30	1.51	1.28	0.90	1.25	1.15	0.99	1.43	0.77	0.94	1.09	1.29	0.97	0.27	1.1
5	1.16	0.27	0.66	0.79	0.46	0.93	1.01	1.10	1.49	1.27	0.74	1.13	1.27	0.88	0.32	0.1
6	1.88	0.38	1.72	1.81	1.53	1.15	1.85	2.07	1.24	1.55	2.11	1.56	2.23	1.73	0.24	1.5
7	2.18	0.29	0.85	1.84	1.75	1.08	1.76	1.21	2.27	1.76	1.60	1.95	2.25	2.02	0.21	1.5
8	1.10	0.23	0.86	0.77	0.92	0.89	0.98	0.44	1.32	1.28	0.89	0.87	1.16	1.18	0.22	0.8
Mean	1.58	0.29	1.17	1.27	0.99	1.12	1.32	1.15	1.56	1.29	1.16	1.15	1.43	1.21	0.30	m =1.1

Table 8

Analysis of variance of growth rates of the eight monokaryon genotypes across all fifteen environments.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios	
Blocks	3	0.1561	0.0520	2.5233	(N.S.)
Environments	14	125.7571	8.9827	189.9091	***†
Genotypes (monokaryons)	7	59.3005	8.4715	182.6754	***†
Environments x Blocks	42	1.9881	0.0473	2.2958	***
Genotypes x Blocks	21	0.9733	0.0464	2.2478	***
Genotypes x Environments	98	69.9600	0.7139	34.6208	***
Genotypes x Environments x Blocks	294	6.1624	0.0210	1.0165	(N.S.)
Between replicates (error)	480	9.8984	0.0206		
Total	959	294.1959			

Significance levels for the above and subsequent analyses:

N.S.	=	P > 0.05 (non significant)
*	=	0.05>P>0.01
**	=	0.01>P>0.001
***	=	P<0.001
†	=	tested against its block interaction

Table 9

Estimates of the genetic, environmental, and genotype-environmental interaction components of variability in growth rates of the eight monokaryon genotypes across all fifteen environments.

Monokaryon Genotype Numbers	Environments															$\hat{d}_i$	b_i
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1	0.30	0.13	0.22	0.25	0.08	0.42	0.10	-0.08	0.04	0.40	-0.37	-0.53	-0.64	-0.40	0.05	-0.09	-0.07
2	-0.08	-0.03	0.15	-0.03	-0.43	-0.30	-0.23	0.14	0.56	-0.27	0.25	0.10	0.21	-0.25	-0.23	0.02	0.04
3	0.22	0.11	0.12	-0.11	-0.06	0.37	0.21	0.06	-0.37	-0.12	-0.11	-0.23	-0.41	0.10	0.19	-0.15	-0.21
4	-0.11	0.10	0.43	0.10	0	0.22	-0.08	-0.07	0.01	-0.43	-0.13	0.03	-0.05	-0.15	0.06	-0.09	-0.13
5	-0.19	0.21	-0.28	-0.25	0.30	-0.04	0.08	0.18	0.16	-0.21	-0.19	0.21	-0.07	-0.10	0.25	-0.23	-0.19
6	-0.11	-0.32	0.14	0.13	0.13	-0.38	0.12	0.51	-0.73	-0.15	0.54	0	0.39	0.11	-0.47	0.41	0.23
7	0.20	-0.40	-0.72	0.17	0.36	-0.44	0.04	-0.34	0.31	0.07	0.04	0.40	0.42	0.41	-0.49	0.40	0.55
8	-0.22	0.20	-0.05	-0.24	0.19	0.03	-0.08	-0.45	0.02	0.25	-0.01	-0.02	-0.01	0.23	0.18	-0.26	-0.22
$\hat{e}_j$	0.45	-0.84	0.04	0.14	-0.14	-0.01	0.19	0.02	0.43	0.16	0.03	0.02	0.30	0.08	-0.83	$\hat{m}$ = 1.13	

genotype are also given. Growth rates across the environments of set 1 are given in Table 10. Table 11 summarizes the analysis of variance for the set 1 data. There are, as before, highly significant differences among the genotypes, and the five temperatures used in the set differ immensely in their effects on performances of the genotypes, as indicated by the high Environments M.S. Interactions between genotypes and temperatures are also highly significant. Estimates of the components of variability and the regression coefficients are given in Table 12. The growth rates over the four dextrose environments of set 2 are listed in Table 13 and the analysis (Table 14) shows that differences in environmental effects caused by the variations in dextrose concentration are highly significant. Differences among genotypes remain prominent, and there are interactions between the genotypes and the concentrations of dextrose which constitute the four environments. Table 15 gives the $\hat{d}_i$'s, $\hat{e}_j$'s, $\hat{g}_{ij}$'s, and b_i's as computed over the set 2 environments. The data from the set 3 environments and their analysis are given in Tables 16 and 17 respectively. Differences among genotypes are clearly indicated and the effectiveness of the casein concentrations in producing environmental variability is unquestionable. The genotypes also demonstrate highly significant interactions with the variations in casein concentration. The three components of variability and the regression coefficients for set 3 are listed in Table 18. Tables 19 and 20 give the set 4 data and corresponding analysis.

Table 10

Growth rates (mean colony diameters in millimeters) of the eight monokaryon genotypes across the five temperature environments (set 1).

Monokaryon Genotype Numbers	Environment Numbers 1	2	3	4	5	Mean
1	1.79	0.33	1.30	1.43	0.98	1.17
2	1.52	0.28	1.34	1.26	0.58	1.00
3	1.65	0.25	1.14	1.01	0.78	0.97
4	1.38	0.30	0.51	1.28	0.90	1.07
5	1.16	0.27	0.66	0.79	0.46	0.67
6	1.88	0.38	1.72	1.81	1.53	1.46
7	2.18	0.29	0.85	1.84	1.75	1.38
8	1.10	0.23	0.86	0.77	0.92	0.78
Mean	1.58	0.29	1.17	1.27	0.99	$\hat{m}$ = 1.06

Table 11

Analysis of variance of growth rates of the eight monokaryon genotypes across the five temperature environments (set 1).

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios
Blocks	3	0.0606	0.0202	1.0790(N.S.)
Environments	4	58.8931	14.7233	288.6922***†
Genotypes (monokaryons)	7	21.2527	3.0361	162.0982***
Environments x Blocks	12	0.6116	0.0510	2.7213***
Genotypes x Blocks	21	0.4414	0.0210	1.1223(N.S.)
Genotypes x Environments	28	14.2270	0.5081	27.1281***
Genotypes x Environments x Blocks	84	1.3515	0.0161	0.8590(N.S.)
Between replicates (error)	160	2.9975	0.0187	
Total	319	99.8354		

Table 12

Estimates of the genetic, environmental, and genotype-environment interaction components of variability in growth rates of the eight monokaryon genotypes across the five temperature environments (set 1).

Monokaryon Genotype Numbers	Environment Numbers					$\hat{d}_i$	b_i
	1	2	3	4	5		
1	0.01	-0.07	0.02	0.05	-0.12	0.11	0.14
2	0	0.05	0.23	0.05	-0.35	-0.06	0.02
3	0.16	0.05	0.06	-0.17	-0.12	-0.09	0.03
4	-0.21	0	0.33	0	-0.10	0.01	-0.07
5	-0.03	0.37	-0.12	-0.09	-0.14	-0.39	-0.35
6	0.70	0.49	0.95	0.94	0.94	0.40	0.24
7	0.28	-0.32	-0.64	0.25	0.44	0.32	0.37
8	-0.20	0.22	-0.03	-0.22	0.21	-0.28	-0.37
$\hat{e}_j$	0.52	-0.77	0.11	0.21	-0.07	$\hat{m}$ = 1.06	

Table 13

Growth rates (mean colony diameters in millimeters) of the eight monokaryon genotypes across the four dextrose environments (set 2).

Monokaryon Genotype Numbers	Environment Numbers				Mean
	1	6	7	8	
1	1.79	1.45	1.33	0.98	1.39
2	1.52	0.84	1.11	1.31	1.20
3	1.65	1.34	1.38	1.06	1.36
4	1.38	1.25	1.15	0.99	1.19
5	1.16	0.93	1.01	1.10	1.05
6	1.88	1.15	1.85	2.07	1.74
7	2.18	1.08	1.76	1.21	1.56
8	1.10	0.89	0.98	0.44	0.85
Mean	1.58	1.12	1.32	1.15	$\hat{m}$ = 1.29

Table 14

Analysis of variance of growth rates of the eight monokaryon genotypes across the four dextrose environments (set 2).

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios
Blocks	3	0.0954	0.0318	1.0315 (N.S.)
Environments	3	8.7223	2.9074	33.0011 ***
Genotypes (monokaryons)	7	18.5151	2.6450	85.8491 ***
Environments x Blocks	9	0.7931	0.0881	2.8601 ***
Genotypes x Blocks	21	0.9683	0.0461	1.4966 (N.S.)
Genotypes x Environments	21	10.1781	0.4847	15.7309 ***
Genotypes x Environments x Blocks	63	2.2980	0.0365	1.1840 (N.S.)
Between replicates (error)	128	3.9438	0.0308	
Total	255	45.5141		

Table 15

Estimates of the genetic, environmental, and genotype-environment interaction components of variation in growth rates of the eight monokaryon genotypes across the four dextrose environments (set 2).

Monokaryon Genotype Numbers	Environment Numbers				$\hat{d}_i$	b_i
	1	6	7	8		
1	0.11	0.23	-0.09	-0.27	0.10	0.21
2	0.03	-0.19	-0.12	0.25	-0.09	0.02
3	0	0.15	-0.01	-0.16	0.07	-0.03
4	-0.10	0.23	-0.07	-0.06	-0.10	-0.46
5	-0.18	0.05	-0.07	0.19	-0.24	-0.67
6	-0.15	-0.42	0.08	0.47	0.45	-0.27
7	0.33	-0.31	0.17	0.21	0.27	1.37
8	-0.04	0.21	0.10	-0.27	-0.44	-0.05
$\hat{e}_j$	0.29	-0.17	0.03	-0.14	$\hat{m}$ = 1.29	

Table 16

Growth rates (mean colony diameters in millimeters) of the eight monokaryon genotypes across the four casein environments (set 3).

Monokaryon Genotype Numbers	Environment Numbers				Mean
	1	9	10	11	
1	1.79	1.51	1.60	0.69	1.40
2	1.52	2.14	1.04	1.43	1.53
3	1.65	1.03	1.02	0.90	1.15
4	1.38	1.48	0.77	0.94	1.14
5	1.16	1.49	1.27	0.74	1.17
6	1.88	1.24	1.55	2.11	1.70
7	2.18	2.27	1.76	1.60	1.95
8	1.10	1.32	1.28	0.89	1.15
Mean	1.58	1.56	1.29	1.16	$\hat{m}$ = 1.40

Table 17

Analysis of variance of growth rates of the eight monokaryon genotypes across the four casein environments (set 3).

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios
Blocks	3	0.0451	0.0150	0.8559(N.S.)
Environments	3	8.2972	2.7658	157.5028***
Genotypes (monokaryons)	7	21.1774	3.0254	80.6773***
Environments x Blocks	9	0.1039	0.0115	0.6572(N.S.)
Genotypes x Blocks	21	0.7884	0.0375	2.1378***
Genotypes x Environments	21	17.2459	0.8212	46.7671***
Genotypes x Environments x Blocks	63	1.1131	0.0177	1:0063(N.S.)
Between replicates (error)	128	2.2481	0.0176	
Total	255	51.0191		

Table 18

Estimates of the genetic, environmental, and genotype-environment interaction components of variability in growth rates of the eight monokaryon genotypes across the four casein environments (set 3).

Monokaryon Genotype Numbers	Environment Numbers				$\hat{d}_i$	b_i
	1	9	10	11		
1	0.21	-0.05	0.31	-0.47	0	0.85
2	-0.19	0.45	-0.38	0.14	0.13	0.36
3	0.32	-0.28	-0.02	-0.01	-0.25	0.14
4	0.06	0.18	-0.26	0.04	-0.26	0.46
5	-0.19	0.16	0.21	-0.19	-0.23	0.11
6	0	-0.62	-0.04	0.65	0.30	-1.96
7	0.05	0.16	-0.08	-0.11	0.55	0.55
8	-0.23	0.01	0.24	-0.02	-0.25	-0.48
$\hat{e}_j$	0.18	0.16	-0.11	-0.24	$\hat{m}$ =	1.40

Table 19

Growth rates(mean colony diameters in millimeters) of the eight monokaryon genotypes across the four yeast environments (set 4).

Monokaryon Genotype Numbers	Environment Numbers				Mean
	1	12	13	14	
1	1.79	0.53	0.70	0.72	0.94
2	1.52	1.27	1.66	0.98	1.36
3	1.65	0.77	0.87	1.16	1.11
4	1.38	1.09	1.29	0.97	1.18
5	1.16	1.13	1.27	0.88	1.11
6	1.88	1.56	2.23	1.73	1,85
7	2.18	1.95	2.25	2.02	2.10
8	1.10	0.87	1.16	1.18	1.08
Mean	1.58	1.15	1.43	1.21	$\hat{m}$ = 1.34

Table 20

Analysis of variance of growth rates of the eight monokaryon genotypes across the four yeast environments (set 4).

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARES	VARIANCE RATIOS
Blocks	3	0.0626	0.0209	0.8054 (N.S.)
Environments	3	7.8657	2.6219	101.2317 ***
Genotypes (monokaryons)	7	38.4684	5.4955	212.1807 ***
Environments x Blocks	9	0.3771	0.0419	1.6178 (N.S.)
Genotypes x Blocks	21	0.5506	0.0262	1.0124 (N.S.)
Genotypes x Environments	21	10.4113	0.4958	19.1421 ***
Genotypes x Environments x Blocks	63	1.5941	0.0253	0.9768 (N.S.)
Between replicates (error)	128	3.3155	0.0259	
Total	255	62.6451		

Table 21

Estimates of the genetic, environmental, and genotype-environment interaction components of variation in growth rate of the eight monokaryon genotypes across the four yeast environments (set 4).

Monokaryon Genotype Numbers	Environment Numbers				$\hat{d}_i$	b_i
	1	12	13	14		
1	0.61	-0.22	-0.33	-0.09	-0.40	1.43
2	-0.08	0.10	0.21	-0.25	0.02	0.11
3	0.30	-0.15	-0.33	0.18	-0.23	0.40
4	-0.04	0.10	0.02	-0.08	-0.16	-0.14
5	-0.19	0.21	0.07	-0.10	-0.23	-0.56
6	-0.21	-0.10	-0.29	0.01	0.51	-0.06
7	-0.16	0.40	0.06	0.05	0.76	-0.98
8	-0.22	-0.02	-0.01	0.23	-0.26	-0.67
$\hat{e}_j$	0.24	-0.19	0.09	-0.13	$\hat{m}$ = 1.34	

Table 22

Analysis of variance of growth rates of the eight monokaryon genotypes across environments 5 and 15 (complete and minimal media).

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARES	VARIANCE RATIOS
Blocks	3	0.0090	0.0030	0.1991 (N.S.)
Environments	1	52.3900	52.3900	3476.4419 ***
Genotypes (monokaryons)	7	3.7296	0.6328	35.3557 ***
Environments x Blocks	3	0.0051	0.0017	0.1121 (N.S.)
Genotypes x Blocks	21	0.3922	0.0187	1.2395 (N.S.)
Genotypes x Environments	7	4.5771	0.6539	43.3889 ***
Genotypes x Environments x Blocks	21	0.3012	0.0143	0.9516 (N.S.)
Between replicates (error)	64	0.9643	0.0151	
Total	127	62.3685		

The differences among genotypes are highly significant and the effectiveness of the different yeast extract concentrations is apparent from the high Environments M.S. There are, in addition, highly significant genotype-environment interactions between the genotypes and the four concentrations of yeast extract. Table 21 summarizes the estimates of the genetic, environmental, and genotype--environment interaction effects, as well as the regression coefficients as assessed across the environments of set 4. The performances of the genotypes in environments 5 and 15 (see Table 7) were subjected to analysis of variance. The results (Table 22) show the ever-consistent significant M.S. for differences among genotypes as well as the highly significant genotype-environment interactions. The unmistakable significance of the difference between environments reflects the inherent difference, in effect on growth rate between the properties of complete and minimal media.

Since all the analyses show significance for differences among genetic effects, and differences among environmental effects as well as the presence of significant genotype-environment interactions, the biometrical-genetical model

$$P_{ij} = \hat{m} + \hat{d}_i + \hat{e}_j + \hat{g}_{ij}$$

may be used to describe the growth rate of each monokaryon genotype in each environment. For example, if we are interested in genotype--temperature relationships, the growth rate of genotype 1 in environment 5 may be represented from the Set 1 data as:

$$P_{15} = \hat{m} + \hat{d}_1 + \hat{e}_5 + \hat{g}_{15}$$

where:

P_{15} = growth rate of genotype 1 in environment 5.

$\hat{m}$ = grand mean growth rate of all genotypes over all environments of set 1.

$\hat{d}_1$ = additive genetic effect of genotype 1 as estimated over all environments of set 1.

$\hat{e}_5$ = additive environmental effect of the 5th environment.

$\hat{g}_{15}$ = interaction effect of genotype 1 with environment 5.

From Tables 10 and 12

$\hat{m}$ = 1.06

$\hat{d}_1$ = 0.11

$\hat{e}_5$ = -0.07

$\hat{g}_{15}$ = -0.12

P_{15} = 1.06 + 0.11 + (-0.07) + (-0.12)

= 0.98

Similarly if we are interested in genotype-casein concentration relationships the growth rate of genotype 2 in environment 9 may be represented from the set 3 data as:

$$P_{29} = \hat{m} + \hat{d}_2 + \hat{e}_9 + \hat{g}_{29}$$

where: P_{29} = growth rate of genotype 2 in environment 9

$\hat{m}$ = grand mean growth rate of all genotypes and environments in set 3.

$\hat{d}_2$ = additive genetic effect of genotype 2 as estimated over all environments of set 3.

$\hat{e}_9$ = additive environmental effect of the 9th environment

$\hat{g}_{29}$ = interaction effect of genotype 2 with environment 9

From Tables 16 and 18

$\hat{m}$ = 1.40

$\hat{d}_2$ = 0.13

$\hat{e}_9$ = 0.16

and $\hat{g}_{29}$ = 0.45

so that P_{29} = 1.40 + 0.13 + 0.16 + 0.45

= 2.14

The model may also be used with those parameters estimated over all fifteen environments. For example, the performance of genotype 8 in environment 6 may be represented as:

$$P_{86} = \hat{m} + \hat{d}_8 + \hat{e}_6 + \hat{g}_{86}$$

P_{86} = mean performance of genotype 8 in environment 6

$\hat{m}$ = grand mean growth rate over all genotypes and environments.

$\hat{d}_8$ = additive genetic effect of genotype 8 as estimated over all environments.

$\hat{e}_6$ = additive environmental effect of the 6th environment

$\hat{g}_{86}$ = interaction effect of genotype 8 with the 6th environment.

From Tables 7 and 9

$\hat{m}$ = 1.13

$\hat{d}_8$ = -0.26

$\hat{e}_6$ = -0.01

$\hat{g}_{86}$ = 0.03

P_{86} = 1.13 + (-0.26) + (-0.01) + 0.03

= 0.89

The regression coefficients which appear in Tables 9, 12, 15, 18, and 21 will be used in subsequent analyses.

(b). Differential response to environmental factors.

The usual incubation temperature for these cells is 22°C, and of the temperatures used in this study, 22°C appears as optimum for seven of the eight genotypes. Genotype 4, however, grows faster at 20°C (Fig. 3). Fig. 4. shows that while the standard 1.0% dextrose concentration of environment 1, appears as optimum for seven of the eight genotypes, the highest concentraction of 1.5% is preferred by genotype 6, while that same concentration is the most adverse to several of the other genotypes. The casein environments of set 3

show a great deal of diversity in response of the genotypes to the various casein concentrations (Fig. 5). The standard 0.5% concentration is optimum for only two genotypes. Five of the genotypes prefer the lowest concentration of 0.05% and one genotype, number 6, grew best at the highest concentration of 1.0%. Genotype 8 showed more growth at both the lowest and intermediate concentrations, than at the standard level. For the yeast extract environments of set 4, the standard concentration of 0.5% proved to be the most suitable for only three genotypes while the highest concentration of 1.0% was more suitable for genotype 8 only (Fig. 6). Genotype 8 also preferred the lower concentration of 0.25% but to a lesser degree. The intermediate concentration of 0.25% as opposed to the standard was more suitable to five of the eight genotypes. The great reduction in growth rate due to the effects of minimal medium (environment 15) is illustrated in Fig. 7. The minimizing effect on phenotypic variability due to environmental extremes is quite evident from the performances in environments 2 and 15, illustrated in Figs. 3 and 7 respectively.

2. Degree of Infection

(a). The parasite genetic effect, the environmental effect and their interaction.

To test the effects of environmental changes without the added complications of having possible influences from differences among host varieties, the sixteen parasite genotypes were grown on the single host cultivar Hannchen, with changes in macro-environmental

Fig. 3. Performances (growth rates) of the eight monokaryon genotypes across the five temperature environments of Set 1.

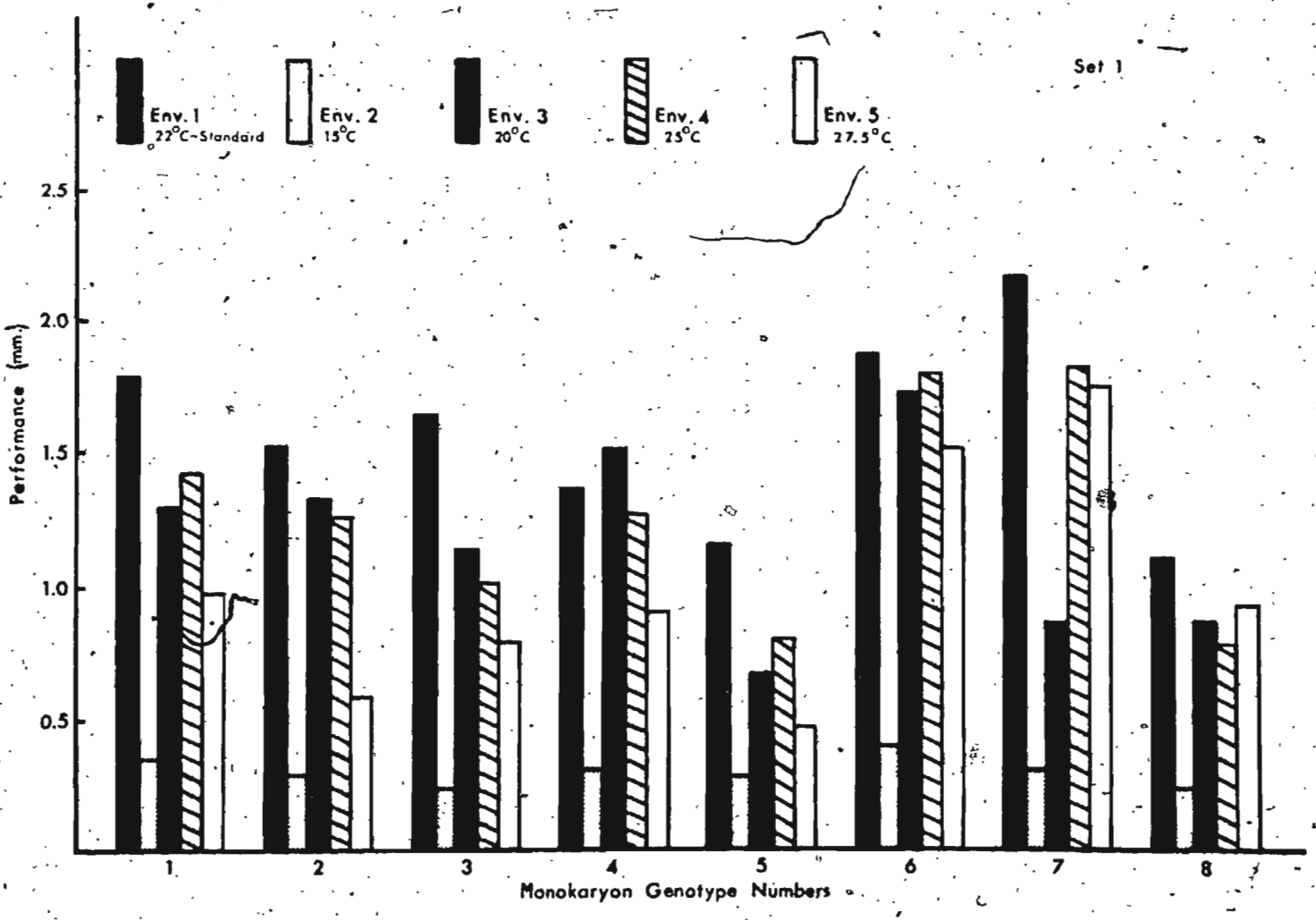

Set 1
Env. 1
22°C-Standard
Env. 2
15°C
Env. 3
20°C
Env. 4
25°C
Env. 5
27.5°C
Performance (mm.)
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
Monokaryon Genotype Numbers

Fig. 4. Performances (growth rates) of the eight monokaryon genotypes across the four dextrose environments of Set 2.

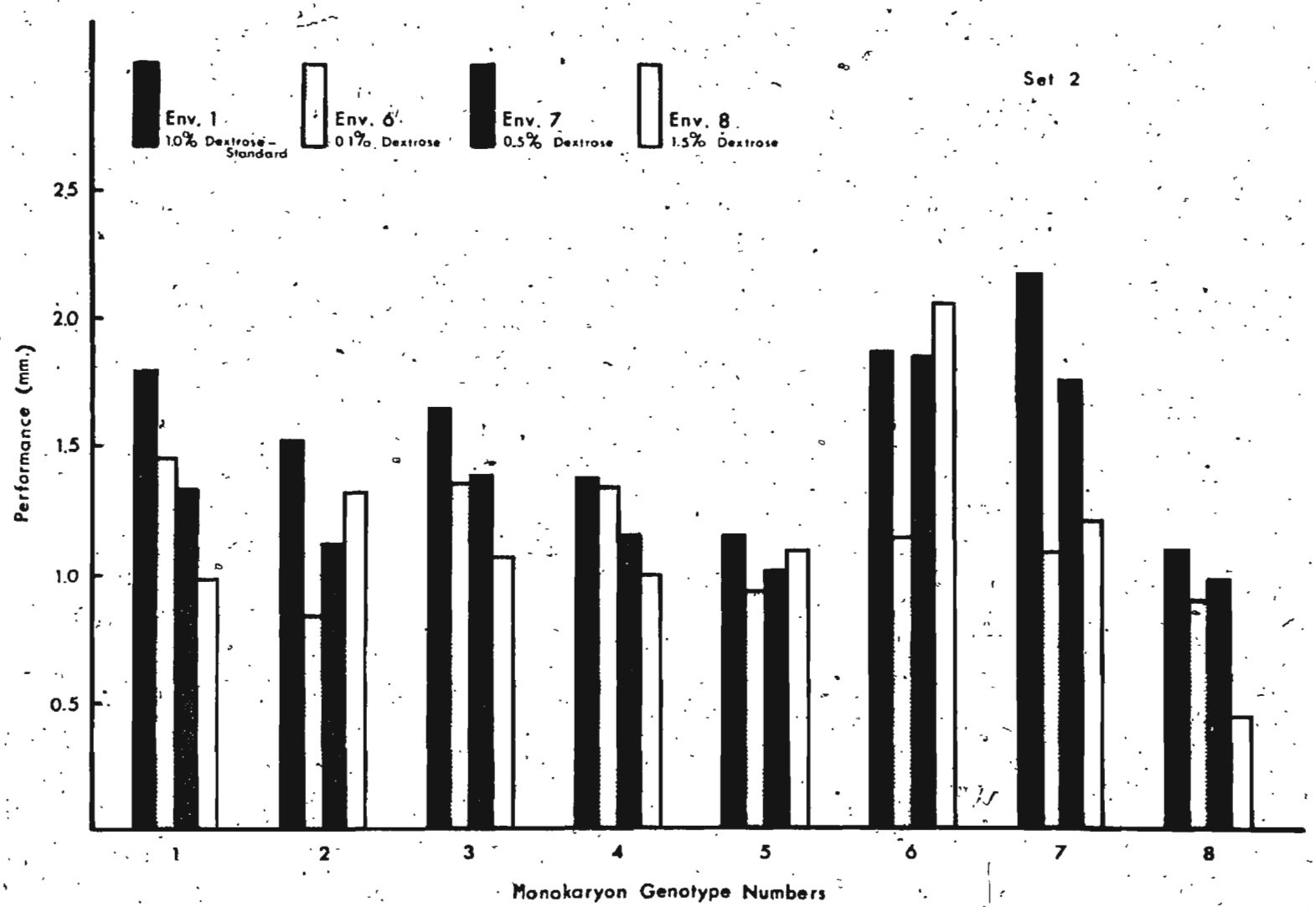

Set 2
Env. 1
1.0% Dextrose – Standard
Env. 6
0.1% Dextrose
Env. 7
0.5% Dextrose
Env. 8
1.5% Dextrose
Performance (mm.)
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
Monokaryon Genotype Numbers

Fig. 5 . Performances (growth rates) of the eight monokaryon genotypes across the four casein environments of Set 3.

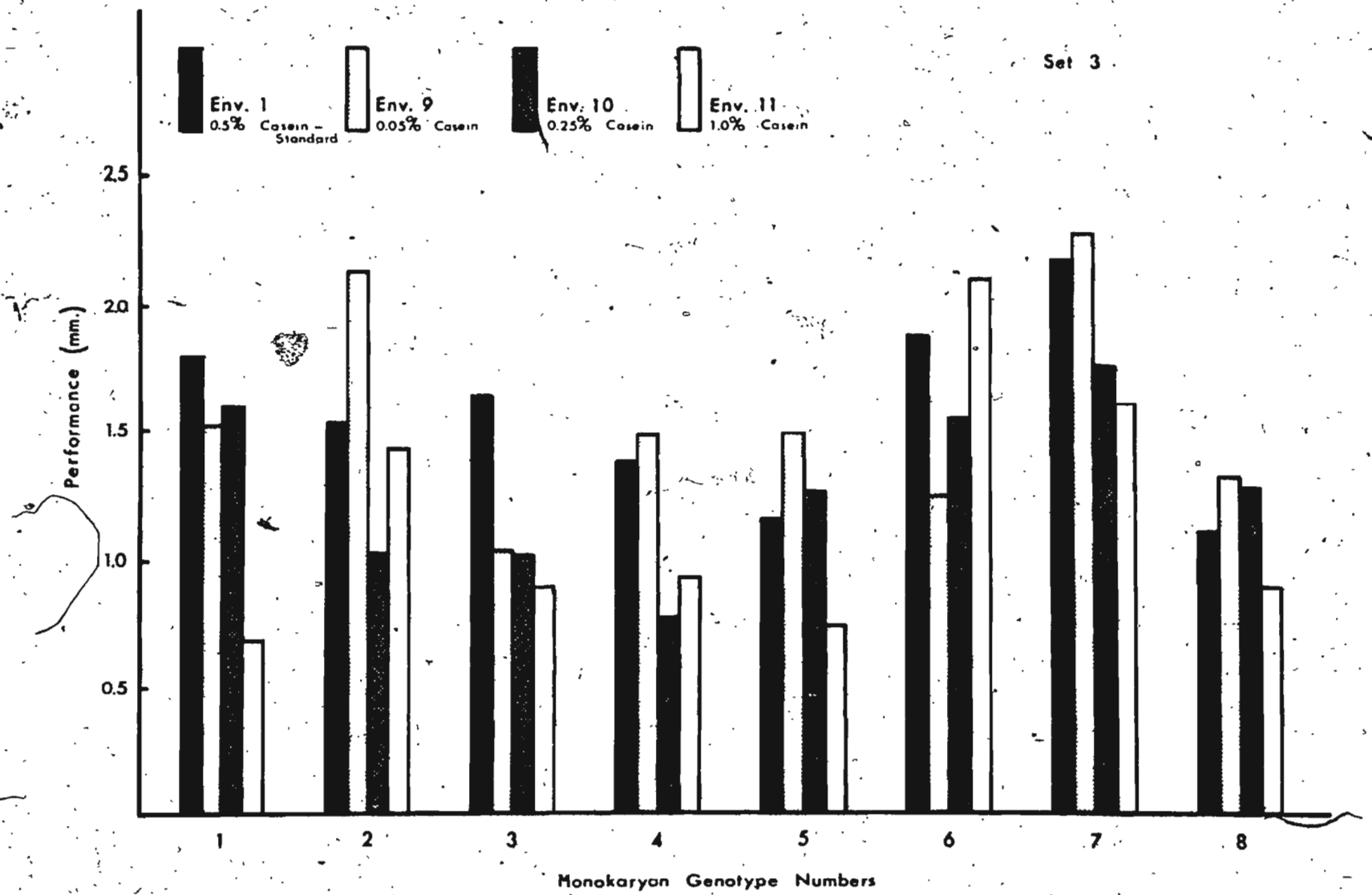

Set 3
Env. 1
0.5% Casein – Standard
Env. 9
0.05% Casein
Env. 10
0.25% Casein
Env. 11
1.0% Casein
Performance (mm.)
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
Monokaryon Genotype Numbers

Fig. 6. Performances (growth rates) of the eight monokaryon genotypes across the four yeast extract environments of Set 4.

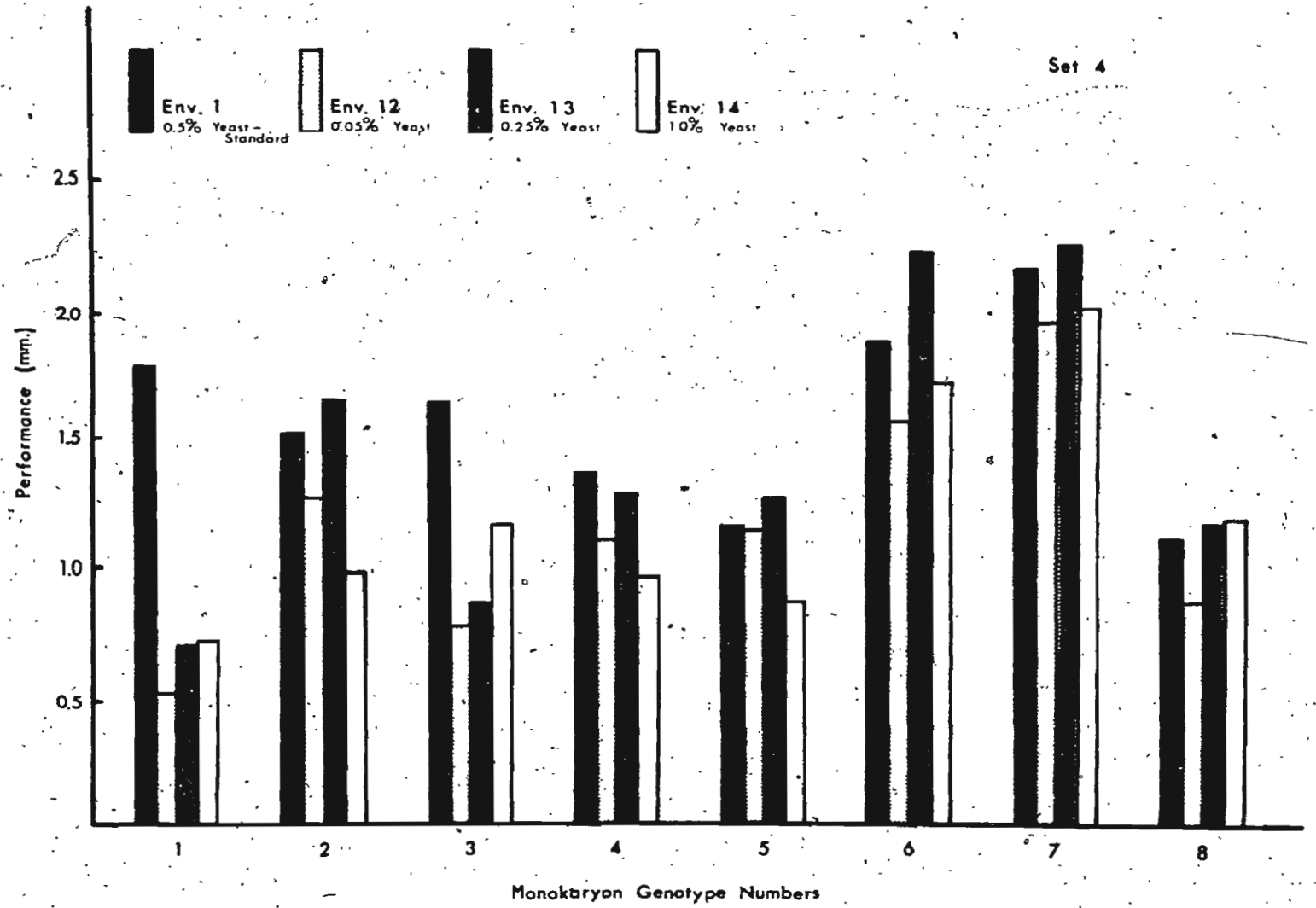
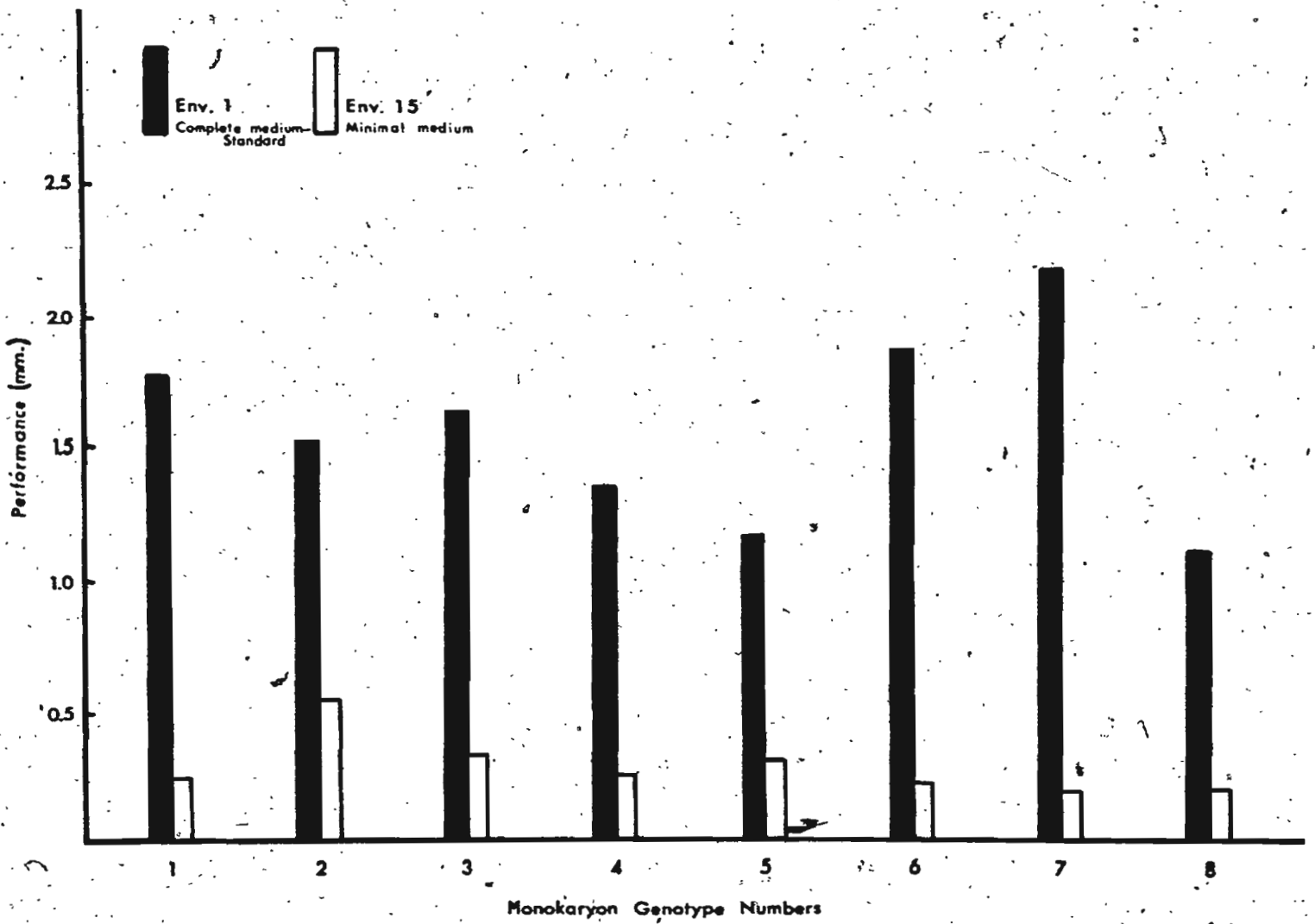

Set 4
Env. 1
0.5% Yeast – Standard
Env. 12
0.05% Yeast
Env. 13
0.25% Yeast
Env. 14
1.0% Yeast
Performance (mm.)
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
Monokaryon Genotype Numbers

Fig. 7. Performances (growth rates) of the eight monokaryon genotypes across environments 1 and 15 (complete and minimal media).


Env. 1
Complete medium-Standard
Env. 15
Minimal medium
Performance (mm.)
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
Monokaryon Genotype Numbers

conditions only (Table 6). The aggressiveness of each parasite genotype in each of the three macro-environments measured as percentage of infection, is given in Table 23. The analysis of variance for the data is shown in Table 24 . Highly significant differences exist among the three macro-environments, and among the sixteen parasite genotypes. However, the Genotypes x Environments M.S. is non-significant, indicating absence of genotype-environment interactions. The genetic effects on aggressiveness ($\hat{p}_i$'s) and environmental effects ($\hat{e}_j$'s) as estimated for the combination of the sixteen parasites and the three environments are ~~given~~ in Table 23.

Components of Variability

Table 25 shows that there are highly significant differences among macro-environments and the effects on variability in aggressiveness of the (+) monokaryons and among the effects of the (-) monokaryons. There are also highly significant genetic interactions between the (+) and (-) monokaryons and it would appear from the relative magnitudes of the variances that the (-) monokaryons contribute more to the variability in aggressiveness than do the (+) monokaryons, but statistically this relationship does not show significance.

The expectation of mean squares (Table 26) provides estimates, firstly, of the amount of total genetic variability in aggressiveness, V_G, due to the (+) and (-) monokaryons

Table 23

Degree of infection of the sixteen parasite genotypes on Hannchen across the three macro-environments.

Parasite Genotype Numbers	Macro-environment Numbers			Mean	p_i
	1	2	3		
1	31.05	59.57	25.07	38.56	-11.21
2	33.45	45.85	20.45	33.25	-16.52
3	65.28	52.78	43.25	53.77	4.00
4	51.65	65.91	46.02	54.53	4.76
5	22.68	46.86	25.69	31.74	-18.03
6	51.05	43.43	29.39	41.29	-8.48
7	43.50	89.58	42.18	58.42	8.65
8	74.13	81.57	35.02	63.57	13.80
9	41.93	50.40	52.31	48.21	-1.56
10	29.83	62.57	25.89	39.43	-10.34
11	49.53	53.73	33.89	45.72	-4.05
12	38.20	49.84	29.27	39.10	-10.67
13	64.15	73.69	61.08	66.31	16.54
14	58.15	66.51	38.38	54.35	4.58
15	57.00	76.39	58.58	63.99	14.22
16	64.80	79.17	48.43	64.13	14.36
Mean	48.52	62.37	38.43		
$\hat{e}_j$	-1.25	12.60	-11.34	m = 49.77	

Table 24

Analysis of variance of the degree of infection of the sixteen parasite genotypes across the three macro-environments.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios	
Blocks	1	406.2738	406.2738	6.3140	*
Environments (macro)	2	3887.7566	1943.8782	30.2101	***
Genotypes (parasites)	15	5100.1355	340.0090	5.2481	***
Environments x Blocks	2	118.4948	59.2475	0.9208	(N.S.)
Genotypes x Blocks	15	1309.7705	87.3180	1.3570	(N.S.)
Genotypes x Environments	30	2492.4076	83.0803	1.2912	(N.S.)
Residual (error)	30	1930.3594	64.3453		
Total	95	15,245.1982			

Table 25

Analysis of variance for further breakdown of the degree of infection of the parasite genotypes across the three macro-environments

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios	
Blocks	1	406.2738	406.2738	6.9534	*
Environments (macro)	2	3887.7566	1943.8782	33.2695	***
(+) Monokaryons	3	1505.9937	401.9978	8.5917	***
(-) Monokaryons	3	2208.9932	736.3311	12.6023	***
(+) Monokaryons x (-) Monokaryons	9	1385.1487	153.9054	2.6341	*
Environments x blocks	2	118.4948	59.2474	1.0140	(N.S.)
(+) Monokaryons x Blocks	3	223.6126	74.5375	1.2757	(N.S.)
(-) Monokaryons x Blocks	3	103.6060	34.5353	0.5911	(N.S.)
(+) Monokaryons x Environments	6	377.1513	63.8586	1.0758	(N.S.)
(-) Monokaryons x Environments	6	287.6186	47.9364	0.8204	(N.S.)
(+) Monokaryons x (-) Monokaryons x Blocks	9	982.5557	109.1728	1.8685	(N.S.)
(+) Monokaryons x (-) Monokaryons x Environments	18	1827.6420	101.5357	1.7378	(N.S.)
(+) Monokaryons x Environments x Blocks	6	241.4397	40.2399	0.6887	(N.S.)
(-) Monokaryons x Environments x Blocks	6	637.2146	106.2024	1.8177	(N.S.)
Residual (error)	18	1051.7070	58.4282		
Total	95	15,245.2083			

Table 26

Expectation of mean squares of Table 25.

Item	Expectation of Mean Squares	Contribution of Item to variability
Environments	$\sigma^2 + rbc\ \Sigma\alpha^2/(a-1)$	58.9203
(+) monokaryons	$\sigma^2 + rac\ \Sigma\beta^2/(b-1)$	18.4821
(-) monokaryons	$\sigma^2 + rab\ \Sigma\nu^2/(c-1)$	28.2467
(+) monokaryons x (-) monokaryons	$\sigma^2 + ra\ \Sigma(\beta\nu)^2/(b-1)(c-1)$	15.9129
(Residual (exp. error; micro-environment)	σ^2	58.4282

Sums of Squares for:

Environments	=	α	r	= Blocks	= 2
(+) monokaryons	=	β	a	= Environments	= 3
(-) monokaryons	=	ν	b	= (+) monokaryons	= 4
			c	= (-) monokaryons	= 4

(the additive genetic component, V_A) and that due to their interaction, (the non-additive genetic component, V_I, due to dominance) and secondly, of the amount of macro--environmental variability, V_E, and micro-environmental variability, V_e). The proportions of the total phenotypic variability, then, contributable to the genetic and environmental components are 35%, 33% and 32% respectively:

V_P	=	V_G	+	V_E	+	V_e
179.9902 (100%)		62.6417 (35%)		58.9203 (33%)		58.4282 (32%)

The additive and non-additive genetic components account for 75% and 25% respectively of the total genetic variability:

V_G	=	V_A	+	V_I
62.6417 (100%)		46.7288 (75%)		15.9129 (25%)

Of the additive genetic component of variability, the (+) and (-) monokaryons contribute 40% and 60% respectively:

V_A	=	$V_A(+)$	+	$V_A(-)$
46.7288 (100%)		18.4821 (40%)		28.2467 (60%)

(b). The parasite and host genetic effects, and their interactions

To test the effects of differences in host reactions without the added complication of influences from environmental changes the sixteen parasite genotypes were grown on five host cultivars (Table 5)

in a common macro-environment (Table 6). The performances (degree of infection) of the sixteen parasites across the five host genotypes are given in Table 27. Analysis of variance (Table 28) shows highly significant differences in aggressiveness among parasites, and the high Host M.S. indicates that the hosts differ immensely in their effects on variability, suggesting different levels of tolerance among the host genotypes. There are no significant interactions between parasites and hosts. The sixteen parasite effects on aggressiveness ($\hat{p}_i$'s) and the five host genetic effects on tolerance ($\hat{h}_k$'s) are given in Table 27. These parameters are merely the more familiar genetic and environmental effects assigned more suitable characters. The difference being that the relationship in this case is between two opposing genetic systems.

Components of Variability.

Table 29 indicates highly significant differences among the host genotypes (i.e. differences in tolerance) and among the effects on variability in aggressiveness of the (+) monokaryons and among the effects of the (-) monokaryons. Genetic interactions between the (+) and (-) monokaryons are still present, and as before, although the variance for the (-) monokaryons is greater than that of the (+) monokaryons, the difference between them is statistically insignificant. The expectation of mean squares in Table 30 provides estimates for the distribution of the genetic components of variability (the aggressiveness of the parasite and tolerance of the hosts) and that portion of the total phenotypic variability due to micro-- environmental effects. The hosts and parasites respectively

account for 34% and 39% of the total variability:

V_P	=	$V_{G(h)}$	+	$V_{G(p)}$	+	V_e
121.3483 (100%)		41.6125 (34%)		47.0637 (39%)		32.6721 (27%)

The additive and non-additive components of genetic variability in aggressiveness, contribute 60% and 40% respectively to the total genetic variabiltiy of the parasites:

$V_{G(p)}$	=	V_A	+	V_I
47.0637 (100%)		28.0541 (60%)		19.0096 (40%)

Of the additive genetic component of variability in aggressiveness, the (+) and (-) monokaryons contribute 46% and 54% respectively:

V_A	=	$V_A(+)$	+	$V_A(-)$
28.0541 (100%)		13.0436 (46%)		15.0105 (54%)

The additive genetic component of variability in aggressiveness ($\hat{a}_i$) for each of the eight monokaryon genotypes is estimated from the performances of the dikaryons averaged over all hosts and environments, measured as deviations from the grand mean, $\hat{m}$ (see Table 31).

Table 27

Degree of infection of the sixteen parasite genotypes across the five host genotypes.

Parasite Genotypes	Host genotypes 1	2	3	4	5	Mean	$\hat{p}_i$
1	25.07	8.14	13.73	9.86	18.50	15.06	-7.56
2	20.45	4.30	5.54	3.63	8.41	8.47	-14.15
3	43.25	24.45	27.44	18.81	23.99	27.59	4.97
4	46.02	26.60	25.19	19.65	38.01	31.09	8.47
5	25.69	7.58	13.93	3.37	23.67	14.85	-7.77
6	29.39	8.89	9.08	6.78	13.95	13.62	-9.00
7	42.18	21.87	27.20	20.91	26.74	27.78	5.16
8	35.02	23.34	9.86	16.52	32.46	23.44	0.82
9	52.31	15.64	13.69	15.62	27.65	24.98	2.36
10	25.89	13.56	23.03	16.05	15.74	18.85	-3.77
11	33.89	8.37	14.07	19.08	15.89	18.25	-4.37
12	29.27	13.72	10.94	3.41	12.31	13.85	-8.67
13	61.08	24.01	21.84	26.04	25.21	31.64	9.02
14	38.38	18.24	15.15	17.06	27.22	23.21	-0.59
15	58.58	39.38	26.41	27.45	40.78	38.52	15.90
16	48.43	17.78	29.86	19.59	37.63	30.66	8.04
Mean	38.43	17.25	17.94	15.24	24.36		
$\hat{h}_k$	15.81	-5.37	-4.68	-7.34	1.64	$\hat{m}$ = 22.62	

Table 28

Analysis of variance of the degree of infection of the sixteen parasite genotypes across the five host genotypes.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios
Blocks	1	18.3951	18.3941	0.5395 (N.S.)
Host genotypes	4	5457.2976	1364.3242	40.0192 ***
Parasite genotypes	15	5576.7351	371.1821	10.8878 ***
Hosts x Blocks	4	56.6800	14.1700	0.4156 (N.S.)
Parasites x Blocks	15	365.3480	24.3656	0.7144 (N.S.)
Parasites x Hosts	60	1477.4319	24.6239	0.7223 (N.S.)
Residual (error)	60	2045.5039	34.0917	
Total	159	14,988.3896		

Table 29

Analysis of variance for further breakdown of degree of infection of the parasite genotypes across the five host varieties

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratios
Blocks	1	18.3737	18.3737	0.5624 (N.S.)
Hosts	4	5457.0921	1364.2730	51.7565 ***
(+) Monokaryons	3	1663.2465	554.4153	16.9691 ***
(-) Monokaryons	3	1899.2795	633.0930	19.3772 ***
(+) Monokaryons x (-) Monokaryons	9	2005.908.	222.7676	6.8183 ***
Hosts x Blocks	4	56.6871	14.1718	0.4338 (N.S.)
(+) Monokaryons x Blocks	3	65.7153	21.9051	0.6705 (N.S.)
(-) Monokaryons x Blocks	3	6.2069	2.0690	0.0633 (N.S.)
(+) Monokaryons x Hosts	12	253.3578	21.1131	0.6462 (N.S.)
(-) Monokaryons x Hosts	12	203.1087	16.9257	0.5180 (N.S.)
(+) Monokaryons x (-) Monokaryons x Blocks	9	293.4502	32.6056	0.9980 (N.S.)
(+) Monokaryons x (-) Monokaryons x Hosts	36	1020.8281	28.3563	0.8679 (N.S.)
(+) Monokaryons x Hosts x Blocks	12	564.4885	47.0407	1.4398 (N.S.)
(-) Monokaryons x Hosts x Blocks	12	304.8918	25.4076	0.7777 (N.S.)
Residual (error)	36	1176.1953	32.6721	
Total	159	14,987.8296		

Table 30

Expectation of mean squares of Table 29.

Item	Expectation of Mean Squares	Contribution of Item to variability
Hosts	$\sigma^2 + rbc\ \Sigma\delta^2/(a-1)$	41.6125
(+) monokaryons	$\sigma^2 + rac\ \Sigma\beta^2/(b-1)$	13.0436
(-) monokaryons	$\sigma^2 + rab\ \Sigma\nu^2/(c-1)$	15.0105
(+) monokaryons x (-) monokaryons	$\sigma^2 + ra\ \Sigma(\beta\nu)^2/(b-1)(c-1)$	19.0096
Residual (experimental error; micro-environment)	σ^2	32.6721

Sums of Squares for:

Hosts = δ

(+) monokaryons = β

(-) monokaryons = ν

r = Blocks = 2

a = Hosts = 5

b = (+)monokaryons = 4

c = (-)monokaryons = 4

Table 31

Mean performances (degree of infection) of the sixteen parasite genotypes over all hosts and environments.

	E_2^+ (2)	E_4^+ (4)	F_2^+ (6)	F_3^+ (7)	Mean	$\hat{a}_i$ (-)
E_1^- (1)	23.70	17.37	36.57	39.00	29.19	-2.84
E_3^- (3)	20.54	23.22	38.85	38.99	30.40	-1.60
F_1^- (5)	31.03	26.67	27.79	22.54	27.01	-4.99
F_4^- (8)	42.29	34.39	46.57	42.47	41.43	9.43
Mean	29.39	25.41	37.45	35.75		
$\hat{a}_i$ (+)	-2.61	-6.59	5.45	3.75	$\hat{m}$ = 32.00	

3. Relationship between Growth Rate and Aggressiveness

The regression coefficients (b_i's) presented in Tables 9, 12, 15, 18, and 21, represent the relative linear sensitivities of each of the monokaryon genotypes to environmental changes, and the deviation mean squares (DMS's) of the individual regression analyses are measures of the non-linear sensitivities to environmental changes (Table 32). (Perkins and Jinks, 1968; Freeman and Perkins, 1971; Fripp and Caten, 1971, 1973).

From the results of the preceeding analyses it is obvious that four aspects of the phenotype of the monokaryons have been dealt with. They are the mean growth rate ($\hat{m}+\hat{d}_i$), the mean aggressiveness ($\hat{m}+\hat{a}_i$), the linear sensitivities to environmental changes (b_i's) and the non-linear sensitivities to environmental changes (DMS's). In addition to mean performances, the sensitivity aspects of the phenotype have also been shown to be under the control of gene systems (Bucio Alanis, Perkins, and Jinks, 1969; Jinks and Perkins, 1970; Paroda and Hayes, 1971; Westerman, 1971 a, b, c). Correlations among such genetic parameters as these have been found in a number of studies (Perkins and Jinks, 1968; Westerman and Lawrence, 1970; Westerman, 1971 a). If any two phenotypic characters are under the control of a common gene system either totally or partially, there will exist between them a significant correlation when these characters are measured over a range of genotypes differing with respect to the two characters. If this correlation cannot be broken by subjecting the genotypes to a series of contrasting environmental conditions, the two characters in

question can be said to share a common gene system (see Fripp and Caten 1973).

Estimates of the four aspects of the phenotype are given in Table 32. For each pair of characters tested, significant positive correlations were found for seven of the eight genotypes. Genotype 8 (F_4) was omitted from the correlation test since it, alone, showed a general trend towards a negative relationship. Between the $(\hat{m}+\hat{a}_i)$'s and $(\hat{m}+\hat{d}_i)$'s only, did these correlations persist across all environmental conditions (see Table 33). Regression lines for the relationship between the $(\hat{m}+\hat{a}_i)$'s and $(\hat{m}+\hat{d}_i)$'s are presented in Fig. 8.

From Table 31 the genetic interaction components of variability in aggressiveness for the sixteen parasite genotypes may be estimated using:

$$\hat{g}_n = \hat{P}_n - \hat{m} - \hat{a}_i^{(+)} - \hat{a}_i^{(-)}$$

where:

$\hat{g}_n$ = effect on variability in aggressiveness, of the genetic interaction between the two parental monokaryon genotypes of the nth parasite.

$\hat{m}$ = grand mean performance (aggressiveness) of all parasites over all hosts and environments.

$\hat{P}_n$ = mean performance (aggressiveness) of the nth parasite over all hosts and environments.

$\hat{a}_i^{(+)}$ = additive genetic effect on variability in aggressiveness of the ith (+) monokaryon genotype.

$\hat{a}_i^{(-)}$ = additive genetic effect on variability in aggressiveness of the ith (-) monokaryon genotype.

The genetic interaction components for the sixteen dikaryons are given in Table 34. The mean performance (aggressiveness) of any parasite measured over all hosts and environments may now be represented as:

Fig. 8. Regression lines for the relationship between $(\hat{m}+\hat{a}_i)$ and $(\hat{m}+\hat{d}_i)$

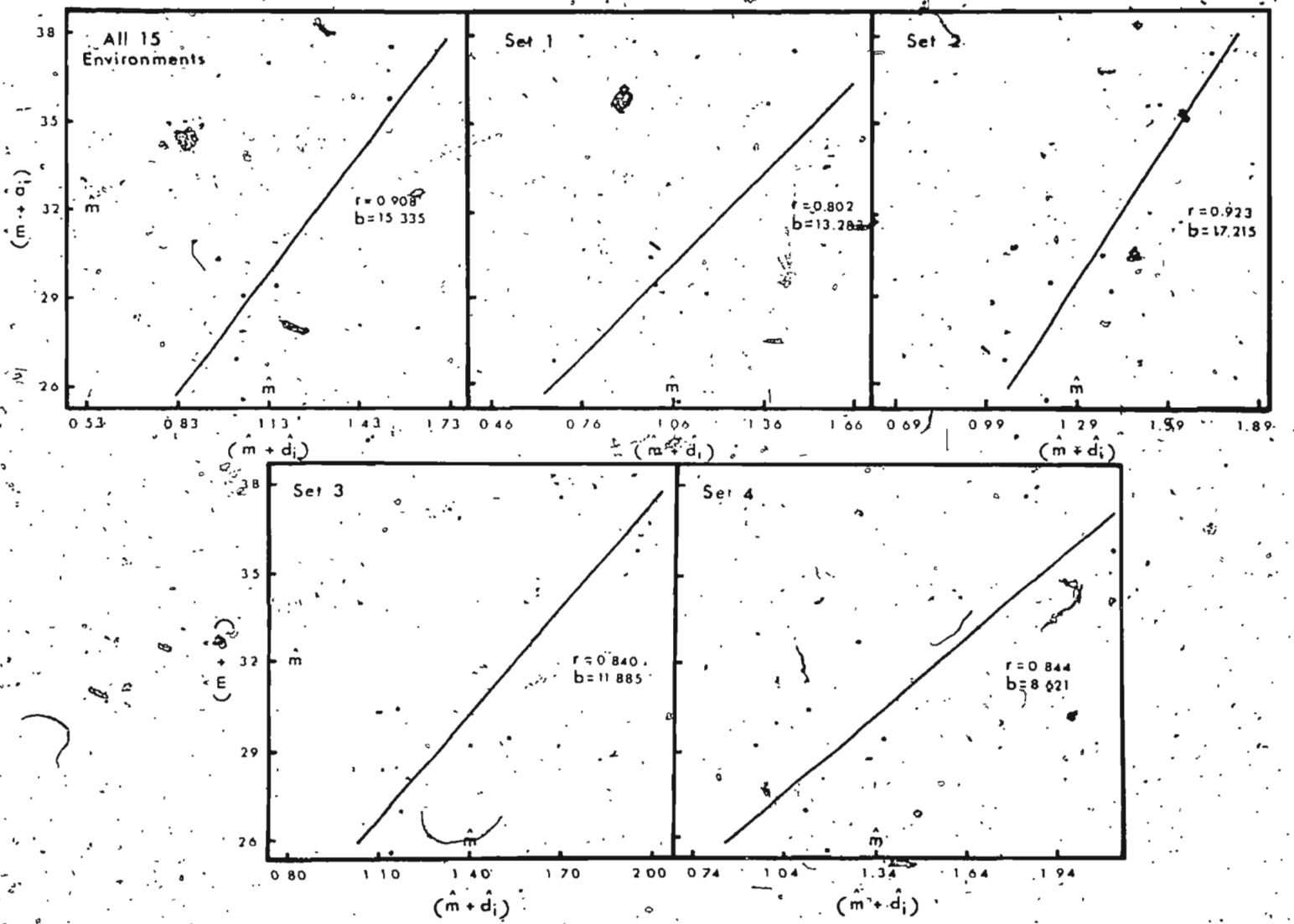

All 15 Environments
r = 0.908
b = 15.335
Set 1
r = 0.802
b = 13.283
Set 2
r = 0.923
b = 17.215
Set 3
r = 0.840
b = 11.885
Set 4
r = 0.844
b = 8.621
$(\hat{m} + \hat{a}_i)$
$(\hat{m} + \hat{d}_i)$
$\hat{m}$

Table 32

The four aspects of the phenotype for the eight monokaryons

Monokaryon Genotype-Numbers	Mean performance (growth rate, $m + \hat{d}_i$)				
	All 15 Environments	Set 1	Set 2	Set 3	Set 4
1	1.04	1.17	1.39	1.40	0.94
2	1.15	1.00	1.20	1.53	1.36
3	0.98	0.97	1.36	1.15	1.11
4	1.04	1.07	1.19	1.14	1.18
5	0.90	0.67	1.05	1.17	1.11
6	1.54	1.46	1.74	1.70	1.85
7	1.53	1.38	1.56	1.95	2.10
8	0.87	0.78	0.85	1.15	1.08
	Linear sensitivities to environmental change ($\hat{b}_i$)				
1	-0.07	0.12	0.21	0.85	1.43
2	0.04	0.02	0.02	0.36	0.11
3	-0.21	0.03	-0.03	0.14	0.40
4	-0.13	-0.07	-0.46	0.46	-0.14
5	-0.19	-0.35	-0.67	0.71	-0.56
6	0.23	0.24	-0.27	-1.96	-0.06
7	0.55	0.37	1.37	0.55	-0.98
8	-0.22	-0.37	-0.05	-0.48	-0.67
	Non-linear sensitivities to environmental changes (DMS)				
1	0.121	0.005	0.070	0.136	0.146
2	0.076	0.060	0.057	0.193	0.061
3	0.048	0.025	0.024	0.089	0.117
4	0.036	0.053	0.021	0.039	0.008
5	0.039	0.022	0.008	0.070	0.029
6	0.132	0.038	0.208	0.158	0.069
7	0.110	0.240	0.014	0.004	0.023
8	0.034	0.019	0.064	0.041	0.024

	Mean performance (aggressiveness, $m+\hat{a}_i$) estimated over all hosts and environments.
1	29.19
2	29.39
3	30.40
4	25.41
5	27.01
6	37.45
7	35.75
8	41.43

Table 33

Correlation values between the aspects of the phenotype for the monokaryon genotypes across all environments and sets.

Aspects of the Phenotype	Correlation values for the Aspects of the Phenotype				
	All 15 Environments	Set 1	Set 2	Set 3	Set 4
$\hat{m}+\hat{d}_i$ and b_i	0.936**	0.935**	0.408(N.S.)	-0.158(N.S.)	-0.512(N.S.)
$\hat{m}+\hat{d}_i$ and *DMS*	0.787*	0.498(N.S.)	0.461(N.S.)	0.120(N.S.)	-0.323(N.S.)
b_i and *DMS*	0.720*	0.594(N.S.)	-0.011(N.S.)	-0.263(N.S.)	0.888**
$\hat{m}+\hat{a}_i$ and b_i	0.805*	0.810*	0.549(N.S.)	-0.612(N.S.)	-0.247(N.S.)
$\hat{m}+\hat{a}_i$ and *DMS*	0.778*	0.487(N.S.)	0.626(N.S.)	0.093(N.S.)	0.084(N.S.)
$\hat{m}+\hat{a}_i$ and $\hat{m}+\hat{d}_i$	0.908*	0.802*	0.923**	0.840*	0.844**

Table 34

The genetic interaction components for aggressiveness, ($\hat{g}_n$) in the sixteen parasites.

	E_2^+	E_4^+	F_2^+	F_3^+
E_1^-	-2.85	-5.20	1.96	6.09
E_3^-	-7.25	-0.59	3.00	4.84
F_1^-	6.63	6.25	-4.67	-8.22
F_4^-	3.47	-0.45	-0.31	-2.71

$$P_n = \hat{m} + \hat{a}_i^{(+)} + \hat{a}_i^{(-)} + \hat{g}_n$$

For example, from Tables 31 and 34 the various parameter estimates for the fourth parasite (E_1F_3) may be substituted:

$$\hat{m} = 32.00$$

$$\hat{a}_1^{(+)} = 3.75$$

$$\hat{a}_1^{(-)} = -2.84$$

and $$\hat{g}_4 = 6.09$$

so that $$P_4 = 32.00 + 3.75 + (-2.84) + 6.09$$

$$= 39.00$$

Assuming, from the results of the correlation tests (Table 33) that a common gene system is controlling variability in both growth rate in the monokaryon and aggressiveness in the dikaryon (parasite), it is not unreasonable to further assume that some idea of the relative performances of the dikaryons can be inferred from measuring the growth rates of the parental monokaryons. That is to say, the mean growth rates of the parental monokaryons should, in some way, indicate the mean aggressiveness of the parasite. The mean growth rates $(\hat{m}+\hat{d}_i)$'s for the eight monokaryons over all fifteen environments are listed in Table 6, and the mean expression of aggressiveness (P_n) for the sixteen parasites are given in Table 31. Since the mean performance across environments is a function of the additive genetic effect, the sum of the $(\hat{m}+\hat{d}_i)$'s for each pair of parental monokaryons was correlated with the P_n of the corresponding dikaryon. A significant

correlation value (r = 0.703**) was found between twelve of the sixteen dikaryons and their parental monokaryones (see Table 35). No correlation was found when using all sixteen dikaryons. However, omitting those dikaryons (13, 14, 15 and 16) containing the monokaryon F_4 (which showed a negative relationship between growth rate and aggressiveness) resulted in a significant positive correlation. The regression line for this relationship is illustrated in Fig. 9. The regression equation which describes the relationship between monokaryotic growth and dikaryotic infection is:

$$P_{n(p)} = 22.84 \left[(\hat{m}+\hat{d}_i)^{(+)} + (\hat{m}+\hat{d}_i)^{(-)} \right] - 23.41$$

where $P_{n(p)}$ = the predicted aggressiveness of the nth parasite.

The performances, observed and predicted, for the twelve parasites are given in Table 36.

4. A Model for Parasite-Host Environment Relationships

Although the dikaryotic parasitic phase of the fungus used in this study showed no interaction with either the environment or host, there frequently does exist such a relationship (Mode, 1958; Van der Plank, 1968), and in cases where interactions contribute a significant portion of variability to the overall level of infestation of a host by a parasite in a certain environment, it would undoubtedly be of immense value to make reliable estimates of the various factors involved in this variability for a better understanding of the effects of possible biological and

Fig. 9. Regression line for the relationship between P_n and $(\hat{m}+\hat{d}_i)^{(+)} + (\hat{m}+\hat{d}_i)^{(-)}$

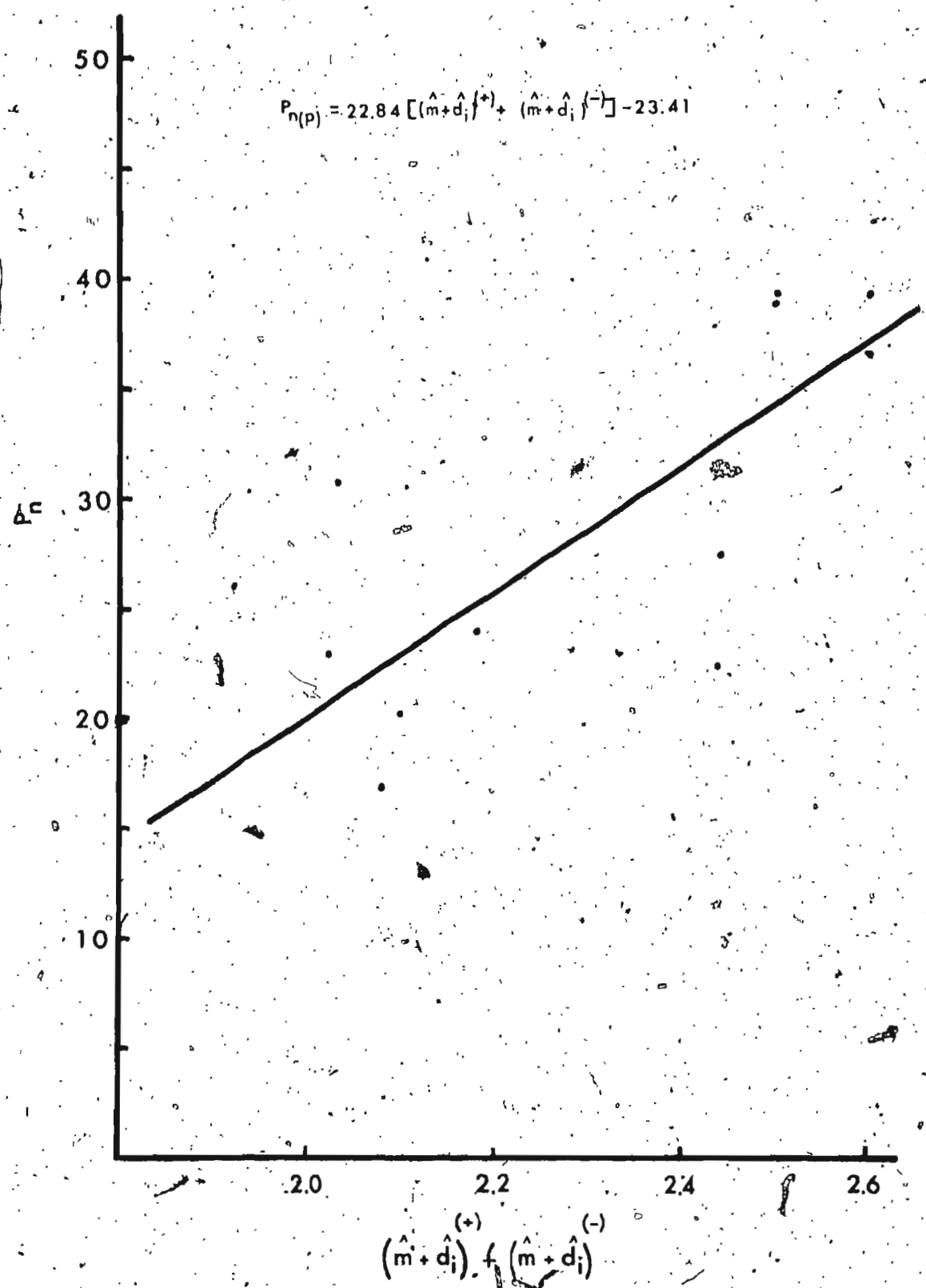

$P_{n(p)} = 22.84\left[(\hat{m}+\hat{d}_i)^{(+)} + (\hat{m}+\hat{d}_i)^{(-)}\right] - 23.41$
50
40
30
20
10
Δ^c
2.0
2.2
2.4
2.6
$(\hat{m}+\hat{d}_i)^{(+)}$ & $(\hat{m}+\hat{d}_i)^{(-)}$

Table 35

The sums of the mean performance (growth rates, $\hat{m}+\hat{d}_i$) of the compatible monokaryons, and the mean performances (aggressiveness, P_n), of the corresponding parasite genotypes.

Parasite Genotype Numbers	$(\hat{m} + \hat{d}_i)^{(+)}$	+	$(\hat{m} + \hat{d}_i)^{(-)}$			P_n
1	1.15	+	1.04	=	2.19	23.70
2	1.04	+	1.04	=	2.08	17.37
3	1.54	+	1.04	=	2.58	36.57
4	1.53	+	1.04	=	2.57	39.00
5	1.15	+	0.98	=	2.13	20.54
6	1.04	+	0.98	=	2.02	23.22
7	1.54	+	0.98	=	2.52	38.85
8	1.53	+	0.98	=	2.51	38.99
9	1.15	+	0.90	=	2.05	31.03
10	1.04	+	0.90	=	1.94	26.67
11	1.54	+	0.90	=	2.44	27.79
12	1.53	+	0.90	=	2.43	22.54

r = 0.703** between $(\hat{m} + \hat{d}_i)^{(+)} + (\hat{m} + \hat{d}_i)^{(-)}$ and P_n

Table 36

The observed (P_n) and predicted ($P_{n(p)}$) performance (aggressiveness) for twelve of the sixteen parasite genotypes.

Parasite Genotype Numbers	P_n	$P_{n(p)}$
1	23.70	26.61
2	17.37	24.10
3	36.57	35.52
4	39.00	35.29
5	20.54	25.24
6	23.22	22.73
7	38.85	34.15
8	38.99	33.92
9	31.03	23.41
10	26.67	20.90
11	27.79	32.32
12	22.54	32.09

physical control measures and their relative efficiencies.

An ideal system for such a study would be composed of (1) a range of parasites varying with respect to aggressiveness. These differences could be due either to minor genotypic distinctions or to higher levels of variation such as those symbolic of species, genera, or populations, (2) a range of environments, being either natural such as geographic locations or seasons, or artifically imposed such as those of the present study, and (3) a range of hosts, again either natural or imposed, which differ in their levels of tolerance. These differences, as with the parasites, may range from minor gene differences to the genetic diversities of whole populations. Observations would then be made on each parasite in combination with each host in each environment.

A model to describe the relationships within such a system would be:

$$I_{ijk} = \hat{m} + \hat{p}_i + \hat{e}_j + \hat{h}_k + (\hat{pe})_{ij} + (\hat{eh})_{jk} + (\hat{ph})_{ik} + (\hat{peh})_{ijk}$$

I_{ijk} = the mean level of infestation (degree of infection, etc) shown by the ith parasite in the jth environment on the kth host.

$\hat{m}$ = the grand mean infestation level measured over all parasites, hosts, and environments.

$\hat{p}_i$ = the genetic effect of the ith parasite (aggressiveness).

$\hat{e}_j$ = the additive effect of the j th environment.

$\hat{h}_k$ = the genetic effect of the kth host (tolerance)

$(\hat{pe})_{ij}$ = the effect of the interaction between the ith parasite and the jth environment.

$\widehat{(eh)}_{jk}$ = the effect of the interaction between the jth environment and the kth host.

$\widehat{(ph)}_{ik}$ = the effect of the interaction between the ith parasite and the kth host.

$\widehat{(peh)}_{ijk}$ = the effect of the interaction among the ith parasite, the jth environment and the kth host.

For t parasites, s environments, and r hosts, the various parameters may be estimated as follows:

$$\hat{m} = I.../tsr$$

(where " . " indicates the mean value taken over the entire range of the missing subscript).

$$\hat{p}_i = I_{i..}/sr - \hat{m}$$

$$\hat{e}_j = I_{.j.}/tr - \hat{m}$$

$$\hat{h}_k = I_{..k}/st - \hat{m}$$

$$\widehat{(pe)}_{ij} = I_{ij.}/r - \hat{m} - \hat{p}_i - \hat{e}_j$$

$$\widehat{(eh)}_{jk} = I_{.jk}/t - \hat{m} - \hat{e}_j - \hat{h}_k$$

$$\widehat{(ph)}_{ik} = I_{i.k}/s - \hat{m} - \hat{p}_i - \hat{h}_k$$

$$\widehat{(peh)}_{ijk} = I_{ijk} - \hat{m} - \hat{p}_i - \hat{e}_j - \hat{h}_k - \widehat{(pe)}_{ij} - \widehat{(eh)}_{jk} - \widehat{(ph)}_{ik}$$

The specification of the model is given in Table 37.

Table 37

The specification for t parasites on r hosts in s environments.

Parasites	Environments 1 — Hosts 1	Environments 1 — Hosts r	Environments s — Hosts 1	Environments s — Hosts r	Sum (Parasites)	Mean (Parasites)
1	$\hat{m} + \hat{p}_1 + \hat{e}_1 + \hat{h}_1 + (\hat{pe})_{11} + (\hat{eh})_{11} + (\hat{ph})_{11} + (\hat{peh})_{111}$	$\hat{m} + \hat{p}_1 + \hat{e}_1 + \hat{h}_r + (\hat{pe})_{11} + (\hat{eh})_{1r} + (\hat{ph})_{1r} + (\hat{peh})_{11r}$	$\hat{m} + \hat{p}_1 + \hat{e}_s + \hat{h}_1 + (\hat{pe})_{1s} + (\hat{eh})_{s1} + (\hat{ph})_{11} + (\hat{peh})_{1s1}$	$\hat{m} + \hat{p}_1 + \hat{e}_s + \hat{h}_r + (\hat{pe})_{1s} + (\hat{eh})_{sr} + (\hat{ph})_{1r} + (\hat{peh})_{1sr}$	$I_{1\cdots}$	$I_{1\cdots}/sr$
t	$\hat{m} + \hat{p}_t + \hat{e}_1 + \hat{h}_1 + (\hat{pe})_{t1} + (\hat{eh})_{1i} + (\hat{ph})_{t1} + (\hat{peh})_{t11}$	$\hat{m} + \hat{p}_t + \hat{e}_1 + \hat{h}_r + (\hat{pe})_{t1} + (\hat{eh})_{1r} + (\hat{ph})_{tr} + (\hat{peh})_{t1r}$	$\hat{m} + \hat{p}_t + \hat{e}_s + \hat{h}_1 + (\hat{pe})_{ts} + (\hat{eh})_{s1} + (\hat{ph})_{t1} + (\hat{peh})_{ts1}$	$\hat{m} + \hat{p}_t + \hat{e}_s + \hat{h}_r + (\hat{pe})_{ts} + (\hat{eh})_{sr} + (\hat{ph})_{tr} + (\hat{peh})_{tsr}$	$I_{t\cdots}$	$I_{t\cdots}/sr$
Sum (hosts within environments)	$I_{\cdot 11}$	$I_{\cdot 1r}$	$I_{\cdot s1}$	$I_{\cdot sr}$	$I\cdots$	$I\cdots/tsr$
Mean	$I_{\cdot 11}/t$	$I_{\cdot 1r}/t$	$I_{\cdot s1}/t$	$I_{\cdot sr}/t$		
Sum (environments)	$I_{\cdot 1\cdot}$		$I_{\cdot s\cdot}$		$I\cdots$	$I\cdots/tsr$
Mean	$I_{\cdot 1\cdot}/tr$		$I_{\cdot s\cdot}/tr$			
Sum (grand)	$I\cdots$					
Mean	$I\cdots/tsr = \bar{m}$					

DISCUSSION

In most quantitative genetic studies to date, little effort has been directed towards examining the effect on phenotypic variability of the interaction between genotype and environment. Only since the late 1960's has interest been shown in the development of biometrical--genetical models and techniques for coping with the problems of making more reliable estimates of genetic, environmental, and interactive effects, as with the difficulties in measuring the sensitivity of genotypes to environmental changes (Bucio Alanis, 1966; Bucio Alanis and Hill, 1966; Perkins and Jinks, 1968; Jinks and Perkins, 1970; Perkins, 1970; Perkins and Jinks, 1971; Fripp and Caten, 1971; Perkins and Jinks, 1973; Fripp and Caten, 1973).

The factors used to create the fifteen growth rate environments (Table 3) were chosen on the basis of their known effects on growth, as well as for convenience, and were not designed to have any physiological implications, as this would be beyond the scope of the present study (see Fripp and Caten, 1971).

It is clear that the growth rates of all the monokaryons were greatly affected by the changes in environments (Table 7). Statistical evidence of this is shown by the significance of the Environments M.S. in Table 8. Further examination of Table 7 shows environments 1, 9, and 13 to be the most stimulating, while 2

and 15 are the most inhibiting.

The results presented in Tables 7 to 22 and Figs. 3 to 7 overwhelmingly show three general facts: (1) environmental changes have a tremendous influence on the growth rates of the monokaryons, (2) variability in growth rate among the monokaryons is extremely high even though they originated from only two teliospores, and (3) the presence of genotype-environment interactions is constant throughout the experiments.

From Tables 7 and 9 it is obvious that monokaryons 6 and 7 have the highest mean performances and the highest $\hat{d}_i$'s, indicating their genetic superiority for growth rate, while monokaryons 5 and 8 are genetically the most inferior. However, this trend is not necessarily indicated if the environments are considered individually, because of variations produced by genotype-environment interactions. It is also evident that the monokaryons having the highest $\hat{d}_i$'s are also those with the highest b_i values, which directly suggest that higher genetic effects are associated with increased linear sensitivities to environmental changes. This relationship is not consistent when considering the individual sets since the sample size represented in each set is drastically reduced. The environmental effects, $\hat{e}_j$'s, indicate that environments 1 (standard) and 9 (0.05% casein) are, generally, the most favourable for the growth of the eight monokaryons. These two environments, differing only in casein concentration, are included in set 3, and represent casein concentrations of 0.5% and 0.05% respectively. Peculiarly, environment 10, having an

intermediate casein concentration of 0.25%, shows a lower effect than either environment 1 or 9. It is clear that the environmental effects are not directly reflected in the performances of the individual monokaryons, and again, this may be explained by the presence of genotype-environment interactions.

Table 38

Variance ratios for the four sets of growth rate environments.

Source of Variation in Growth rate	Set 1 (temperature)	Set 2 (dextrose)	Set 3 (casein)	Set 4 (yeast extract)
Environments	(4) 288.6922	(3) 33.001	(3) 157.5028	(3) 101.2317
Monokaryon Genotypes	(7) 162.0982	(7) 85.8491	(7) 80.6773	(7) 212.1807
Genotype--Environment Interaction.	(28) 27.1281	(21) 15.7309	(21) 46.7671	(21) 19.1421

Degrees of freedom are shown in parentheses

From Table 38 the variance ratios may be used as indications and comparisions of the amounts of variability within the four environment sets. Firstly, among the environments, the greatest effect on variation in growth rate is due to differences in temperatures; the next largest effect on the variation is due to differences in casein concentrations, followed by the yeast extract concentrations and finally by the dextrose concentrations. Secondly, for the monokaryons, it is clear that the most variability appears among them when subjected to differences in yeast extract concentrations. Less variability is shown under the different temperatures, lesser still under the dextrose concentrations, and the least under the casein concentrations. Thirdly, for

genotype-environment interaction, the greatest effect on variability is due to the interaction of the monokaryon genotypes with the casein concentrations, followed by progressively smaller effects due to interactions with temperatures, yeast extract concentrations, and dextrose concentrations, respectively.

Whenever genotype-environment interactions are present, the phenotypic differences among genotypes will change when the genotypes are subjected to contrasting environmental factors. This may be illustrated quite easily. Firstly, by reference to Fig. 3. the phenotypic difference between the genotypes 7 and 8, when grown in environment 3 (20°C), is only 0.01 mm., but the same two genotypes when subjected to the conditions of environment 4 (25°C), show a greatly increased difference of 1.07 mm. Secondly, from Fig. 5. it can be seen that while genotype 1 grows better than genotype 2 in environment 10 (0.25% casein), the situation is reversed in environment 11 (1.0% casein). Thirdly, Fig. 6. shows that, while environment 1 (0.5% yeast) is better than environment 13 (0.25%) for genotype 1, the reverse is true for genotype 6. From Table 7 it is obvious that environment 1 gives the highest mean growth rate (1.58 mm.) over all the genotypes. It, then would be a logical choice as an optimum environment for the growth of these genotypes. However, because of genotype-environment interaction, some of the genotypes show growth rates in this environment much less than those found in the more adverse environments. It is for this reason that environment 1 has been referred to as the "standard" environment and not the "optimum". Strictly speaking, the optimum environment would be one in which all conditions were the most suitable to all the genotypes. This of course, would be impossible whenever genotype-environment interaction is present. It is quite likely that an optimum environment would have to be proposed

for each genotype if the best phenotypic expression is to be achieved. This type of approach is quite practical and economical in developing environment-specific varieties of plants and animals.

If a genotype displays variation in its performance through interaction with its environment, it is also displaying relative instability. That is to say, the more stable the genotype, the less it will exhibit genotype-environment interaction, and its performance will remain relatively constant over a range of environments. The most stable genotypes are those which show no genotype-environment interactions at all. Variations in individual performances by such genotypes will be due solely to environmental influences.

One way to estimate genotypic stability is to compare the linear sensitivity values (b_i's) of the genotypes involved. Higher b_i's would indicate more phenotypic variability, and the lower b_i's less variability. Likewise, comparison of the non-linear sensitivity values (DMS's) would provide similar estimates. The sensitivity values are summarized in Table 32. Table 33 shows that these values are significantly correlated (r = 0.730*) based on estimates made over all fifteen environments and suggests that the proportions of linear and non-linear sensitivities tend to remain constant from genotype to genotype. Both types of sensitivity estimates are, in turn, significantly correlated with the mean growth rates ($\hat{m}+\hat{d}_i$) when all fifteen environments are considered. The temptation here may be to conclude that these three aspects of the phenotype may be partly controlled by a common gene system. Such a relationship is frequently found to exist (Perkins and Jinks, 1968 a; Westerman and Lawrence, 1970; Westerman, 1971 a). However,

the relationship in this case is not consistent, and breaks down under the different sets of environmental conditions, suggesting, in fact, that separate gene systems may be controlling these characters (Perkins and Jinks, 1968 b; Westerman and Lawrence, 1970; Paroda and Hayes, 1971).

Recently, Easton and Clements (1973) indicated the use of stability estimates based on the sums of the squared values of the genotype--environment interactions for each genotype:

$$\hat{S}_i = \sum_j \hat{g}_{ij}^2$$

where $\hat{S}_i$ = a measure of the stability of the ith genotype

$\hat{g}_{ij}$ = interaction estimate for the ith genotype with the jth environment.

Table 39.

The stability estimates for the eight monokaryon genotypes over all 15 environments.

Monokaryon Genotype Numbers	Stability estimates: $\hat{S}_i = \sum_j \hat{g}_{ij}^2$
1	1.5685
2	0.9966
3	0.7057
4	0.5081
5	0.5788
6	1.8189
7	2.0349
8	0.5432

Based on the concepts implied by the formula, the genotype having the lowest $\hat{S}_i$ is the most stable while the higher $\hat{S}_i$'s reflect the less stable genotypes. High stability does not necessarily mean high performance (Bucio Alanis, 1966); in fact, the reverse situation can occur as indicated in Table 39 which gives results generally in agreement with those shown by the b_i's and *DMS*'s in Table 32. Genotypes 6 and 7, having the highest mean performances of 1.54 mm. and 1.53 mm. respectively (see Table 7) are the least stable, while genotype 8 having the lowest mean performance (0.87 mm.), is the second highest in terms of stability. It should be remembered that these stability estimates are relative to those genotypes and environments used, and will not necessarily represent the same levels of stability when compared to those of other genotypes or in other environments. The fact that the fastest growing cells appear to be those having the least stable genotypes, suggests that genotype-environment interactions may play a very important role in the survival and possible competition of these cells in their natural environment.

Following dikaryotization there is a complete change in the response of the genotypes to environmental factors. The individual monokaryon genotypes when mated show no sign of interaction with the environment, and each dikaryon thus formed is highly stable in its genetic control over aggressiveness, displaying total absence of genotype--environment interaction and also showing a complete lack of interactions with the host. A better understanding of genotypic stability may lie behind the reason for the dramatic increase in stability of the genome of this fungus once the dikaryotic phase is reached. Such a find would

obviously be of immense value in breeding programs, since any increased ability to produce stable genotypes could lead to a faster and more efficient method of breeding varieties capable of showing a uniform, and hence predictable, performance under all possible environmental conditions.

Information about the non-parasitic phase of *U. hordei* is important in its own right, but what may be of more direct significance is the relationship between the non-parasitic and parasitic phases of this pathogen. The main objective for studying certain aspects of the growth rate of the monokaryon was to attempt to uncover some kind of genetic correlation between it and aggressiveness in the dikaryon. This study of aggressiveness is centered around the investigation of the genetic and environmental aspects of host-parasite relationships in terms of measures of infection. The reason for studying degree of infection is the interest in the parasite's aggressiveness and the host's tolerance (horizontal resistance) which is usually controlled by polygenes and is much more lasting than vertical resistance controlled by a small number of major genes (Van der Plank, 1968). This is, of course, of great significance in controlling diseases of economically important plants and animals. Another reason for studying aggressiveness includes the probability that understanding this character in the parasite may indicate ways of overcoming it, and may present the possibility of developing highly aggressive strains of parasites for use in biological control.

Degree of infection in any host-parasite system can be affected by the genotypes of the parasite(aggressiveness) and host (tolerance) and by

the environment in which they co-exist. Table 23 shows that all sixteen parasite genotypes are capable of producing infection. The effect of the macro-environments as well as the effect of the parasite genotypes, in producing variation in the degree of infection, is undeniably significant, as supported by the results of the analysis of variance (Table 24). Variability in aggressiveness within each environment is obvious and appears continuous as noted by Emara and Sidhu (1974) using identical parasite genotypes on the host cultivar Vantage. This variability is directly reflected in the parasites' mean aggressiveness and their genetic effects, $\hat{a}_i$'s. Variability between environments, across each parasite is reflected in the differences between environment means and environmental effects, $\hat{e}_j$'s: With $\ell.s.d.$ = 7.80 at 5% these environment means are all significantly different from one another, confirming that environment 2 provided the most suitable conditions for infection, followed by environment 1 and lastly by environment 3. Apart from the differences in soil conditions between the field experiment and the two greenhouse experiments, the main differences among the three environments are among their temperatures. The mean temperature during the growing period of environment 1 was 15°C with fluctuations ranging from 7°C to 40°C. Environment 2 had a mean temperature of 16°C with fluctuations from 12°C to 20°C, and environment 3 had a mean temperature of 12°C with fluctuations from 2°C to 28°C. These temperature readings help to support the claim that environment 2 was the most favourable for the fungus, since they are in agreement with the findings of Faris (1924) who obtained the highest percentages of covered smut infection between 15°C and 20°C. Temperatures above and below this range hindered the growth and development of the fungus.

The lack of genotype-environment interactions (Table 24) suggests that the relative aggressiveness of the parasite genotypes should be the same in the three environments. This would have been so if strict control could have been enforced over all conditions of the experiment. This, however, is an impossible task when using greenhouse and field facilities, and the result is the existence of experimental error attached to the percentages of infection, making them higher or lower than their true values. However, the fact remains that these parasite genotypes are stable in relation to each other in these three environments, and they may still exhibit stability over a wider range of environments, but this has yet to be demonstrated. One of the advantages of stability is that the results obtained from an experiment in one environment, concerning the relative aggressiveness of a group of parasite genotypes, will be expected to hold true in other environments, with changes due only to the physical effects of environmental conditions. The importance of the environment in the variation in degree of infection is realized from the analysis of variance (Table 25) and subsequent expectations of mean squares (Table 26). While genetic factors account for 35% of the variability, macro-environmental influences are responsible for 33% and the micro-environment, 32% of the variability.

The analysis of variance in Table 28 clearly indicates that the only significant items in Table 27 are the differences in tolerance among the five host genotypes, and the differences in aggressiveness among the sixteen parasite genotypes. With $\ell.s.d.$ = 8.44 at 5% it is evident that host genotype 1 (Hannchen) is significantly less tolerant than the other four, and that 5 (Odessa) is less tolerant than 4 (Trebi), while there is no significant difference among 2 (Vantage), 3 (Lion) and 4 (Trebi),

or among 2, 3, and 5. There is no interaction between hosts and parasites which indicates relative stability of the tolerance of the host genotypes, as well as stability in aggressiveness of the parasites. This should mean that when exposed to parasite genotypes other than those used in this study, the hosts will produce the same ranking (relative to one another) regarding their tolerance. Similarly, the parasites should retain their same relative rankings regarding aggressiveness, when confronted with other sets of host genotypes. Examination of Table 27 shows that host 1 (Hannchen) is consistently the least tolerant towards all sixteen parasites, and that parasite 2 is the least aggressive on four of the five hosts, with the one exception (host 4) within the variation due to experimental error. Parasite 15 is clearly the most aggressive of all. Again, this type of genotypic stability may make the results obtained, applicable to other genotypes of hosts and parasites. From the analysis of variance (Table 29) and the expectations of mean squares (Table 30) it is evident that the hosts are a significantly contributing factor in the variation shown in degree of infection, displaying a contribution of 34%.

As mentioned earlier, it was hoped, from data collected from this study, to find a genetic correlation between growth rate and aggressiveness. Since the study of aggressiveness requires a great deal of time and space, such a correlation would provide comparable information simply from the study of growth rate under controlled laboratory conditions.

The correlations between mean growth rate ($\hat{m}+\hat{d}_i$) and mean aggressiveness ($\hat{m}+\hat{a}_i$) would be identical to correlations between the genetic effect on growth rate ($\hat{d}_i$) and the genetic effect on aggressiveness ($\hat{a}_i$) since

the $\hat{m}$'s in each case are constant. The significance of the relationship between growth rate and aggressiveness is shown in Table 33 and Fig. 8., and is constant regardless of the environmental conditions used. The persistence of such a high correlation between the genetic control of growth rate and aggressiveness suggests that both characters may be influenced by a common gene system. Since these characters are under polygenic control, it is unlikely they would share equally all the genes in the system. Instead, a more reasonable relationship would be to show partial sharing of all the available genes. The coefficients of determination (r^2)'s for the values given in the last row of Table 33 show that the percentage of genes in common between the two characters in question, ranges from approximately 64% to 85% depending on the environmental conditions (Table 40).

Table 40

Percentage of genes in common for Growth rate and Aggressiveness.

All 15 Environments	Set 1	Set 2	Set 3	Set 4
82.46	64.27	85.17	70.63	71.18

The differences among the percentages may be due to the presence of environment specific genes, i.e. genes which are expressed or are more active, only under specific environmental conditions.

The correlation between the mean aggressiveness of the parasite genotypes (P_h) and the sum of the mean growth rates of their parental monokaryons, $(\hat{m}+\hat{d}_i)^{(+)} + (\hat{m}+\hat{d}_i)^{(-)}$, was highly significant ($r = 0.703^{**}$).

The main difference between this correlation and those previous is that $P_n = \hat{m} + \hat{a}_i^{(+)} + \hat{a}_i^{(-)} + \hat{g}_n$. The last term ($\hat{g}_n$) in the expression represents the genetic interaction between the (+) and (-) monokaryons. This factor has no equivalent in the monokaryon growth rate expression, so the reduction in the magnitude of the correlation coefficient is expected.

It should be remembered, however, that the material used in this study is a very small portion of the total number of possible genotypes, and therefore cannot be regarded as a representative sample. There is the possibility that the correlations could have arisen by chance alone since only seven of the eight monokaryon genotypes showed this relationship, and reliable predictions could be made for only the twelve dikaryon genotypes in which the eighth monokaryon is not involved. Moreover, we have to realize that the eighth monokaryon is closely related to three of the other monokaryons since they constituted a tetrade derived from a single diploid teliospore. This suggests that there is segregation from this diploid of genes which affect aggressiveness while having little effect on growth rate. Whether or not the close association between monokaryotic growth and dikaryotic aggressiveness has been brought about by chance or by pleiotropy, could be determined by further examination. If it is found that the relationship continues over a large sample of genotypes, the predictive quality of the relationship would be enhanced, and similar analysis could be applied to other pathogens.

As stated earlier, conditions often arise whereby interactions within a parasite-host-environment system, become prominent. If it is found

that such interactions are taking place, i.e. changes in aggressiveness of certain forms of the pathogen with changes in the host varieties or environments, or changes in the tolerances of varieties due to changes in environments, a model, such as the one described in this work could be used to characterize these interactions. If, for example, tolerances change with environments and pathogens, it could well be found that the variety which shows the highest tolerance over all environments and pathogen genotypes, does not have the highest tolerance in a specific environment or when confronted with a specific pathogen genotype. A variety with a normally low tolerance could prove more suitable for use in these cases. The greatest benefit of having a model to describe these relationships lies in its ability to be selective. That is, the model can choose the variety exhibiting highest tolerance, disregarding environments and pathogens, or it may select the most tolerant variety with respect to a specific environment, or to a specific pathogen genotype. The model will also indicate the variety most tolerant to each pathogen genotype in combination with each environment. These same concepts may be applied to the testing of aggressiveness in certain pathogen genotypes which could well show variation through interactions with hosts and environments.

SUMMARY

1. Eight monokaryon, and sixteen dikaryon, genotypes of *Ustilago hordei* (Pers.) Lagerh. were exposed to a variety of environmental conditions and host genotypes.

2. The analysis of data from the monokaryon experiments showed that rate of growth in the haplo-phase of *U. hordei* is controlled by a polygenic system, and that there is a high incidence of genotype-environment interaction.

3. Degree of infection was found to be affected by the aggressiveness of the parasite (dikaryon) genotype, the tolerance of the host genotype, and the environment.

4. The parasite genotypes were shown to vary significantly in aggressiveness, and there were highly significant differences among environments. There were, however, no parasite-environment interactions, indicating high genotypic stability in the parasites.

5. Highly significant differences were found among the tolerances of the host genotypes and these differences remained relatively constant over the range of parasites used, due to the lack of parasite-host interactions.

6. A relationship was found between genetic control of growth rate in the monokaryons and genetic control of aggressiveness in the dikaryons derived from them, and statistically valid predictions of infectivity were made, based on this relationship.

7. A mathematical model to describe parasite-host-environment relationships is presented with reference to its possible areas of use.

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