

# FRUITING AND GROWTH RATE AMONG DIKARYOTIC PROGENY OF SINGLE WILD ISOLATES OF *SCHIZOPHYLLUM COMMUNE*

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Received January 17, 1966

NATURALLY occurring genetic variation has been demonstrated in several species belonging to the Hymenomycetes, as well as in other fungi. The best known examples are of course the incompatibility factors found in the heterothallic species (WHITEHOUSE 1949). Other major genes segregating among progenies of wild dikaryons were found to affect the shape of the fruit bodies (ZATTLER 1924; RAPER and KRONGELB 1958; JÜRGENS 1958; KIMURA and FUJIO 1961) and the morphology of monokaryotic mycelia (CROFT and SIMCHEN 1965).

As in higher plants and animals, most heritable wild variation in fungi, however, is continuous and highly sensitive to environmental conditions; it is therefore more difficult to detect than major gene differences. Such heritable variation has been shown to exist for growth rate of monokaryons in *Collybia velutipes* (CROFT and SIMCHEN 1965) and in *Schizophyllum commune* (SIMCHEN 1966). Demonstration of heritable variation for dikaryotic traits requires the mating of monokaryotic lines, and is therefore not as simple as the demonstration of monokaryotic variation. KNIEP (1928) has already suggested that the ability of the Hymenomycetes to fruit is determined by the genotype, although not necessarily by major genes. GILMORE (1926), BRODIE (1948), BARNETT and LILLY (1949) and RAPER and KRONGELB (1958) have in fact shown experimentally that fruiting ability is controlled by the genotype in a way that cannot be explained by simple major gene models.

Investigations of continuously varying characteristics require suitable genetical tools, which differ from those used to investigate single gene differences. Suitable biometrical methods have been developed in our laboratory, and used to investigate the genetic control of dikaryotic growth rate among progenies of two wild isolates of *S. commune* (SIMCHEN and JINKS 1946). The same methods are used here to obtain further information about the determination of growth rate of dikaryons in this fungus, as well as to investigate the genetical control of other characters, such as fruiting time and fruit weight.

## MATERIALS AND METHODS

Six dikaryons were isolated from dry fruit bodies recently collected in the wild. Isolates 1 and 2 were obtained from dry fruit bodies collected in England (SIMCHEN and JINKS 1964), and isolates 3,4,5 and 6 from fruit bodies collected in Massachusetts, U.S.A. (SIMCHEN 1966). The methods of isolation, fruiting and the raising of progeny are also given in the publications cited above. The mating types of the monokaryotic progeny and of the original dikaryotic isolates were

determined following PAPAŽIAN (1950) as described by SIMCHEN (1966). Thus the monokaryotic progeny of each of the six isolates were classified into four major groups according to their mating types:  $(A1B1 + A2B2) \rightarrow A1B1, A1B2, A2B1, A2B2$ . These could be mated to form dikaryons in two combinations only:  $A1B1 \times A2B2$  and  $A1B2 \times A2B1$ . Six progeny were chosen at random from each of the four groups of monokaryons, and all possible matings were made between them, so that every monokaryon of mating type  $A1B1$  was mated with six  $A2B2$  monokaryons, and so on. Thus among the progeny of each dikaryon, two  $6 \times 6$  dikaryotic combinations were formed, to give 72 dikaryotic descendants derived from all compatible combinations among 24 monokaryons. This experimental design is shown diagrammatically by SIMCHEN and JINKS (1964, Figure 1): the four sets of monokaryotic progenies are designated *a-f*, *g-l*, *m-r*, *s-z*, and the two sets of dikaryotic combinations are  $(a-f) \times (g-l)$  and  $(m-r) \times (s-z)$ . Hence the latter two sets are genuinely independent and are duplicate samples of the dikaryotic progeny of the isolate under investigation.

Quantitative characters, such as growth or fruiting, can be measured in several ways. In the experiments reported here, it was the aim to use metrics that are easy to measure, are subject to small errors within an experiment, and can be compared between different experiments with some confidence.

*Growth rate* was determined in growth tubes at 25°C on MT medium, as described by SIMCHEN and JINKS (1964) and CROFT and SIMCHEN (1965). Each experiment consisted of four randomised blocks, each block being confined to a shelf in the incubator. Every block contained 97 growth tubes, that is one tube each of the original dikaryon, the 24 monokaryons and the 72 dikaryons. The mycelial growth in the tubes was marked three days after inoculation, and measurements (in mm) were taken ten days later. During this period the rate of growth was found to be constant; the ten days growth is therefore referred to throughout as "growth rate."

*Fruiting time*. Experiments in which fruiting characters were scored were carried out in conditions as uniform as possible: portions of 25 ml of SF medium were poured into sterile Petri-dishes (9 cm in diameter) through a medium dispenser (for the composition of media see SIMCHEN and JINKS 1964). Dikaryons grown on SC medium were used as sources of inocula, which were 1 to 2 mm in size and were placed in the centres of the SF plates. The Petri-dishes also contained disks of filter paper (Whatman No. 1) in order to prevent condensation of excess water on the lids of the dishes. The inoculated plates were placed in the coldroom ( $18 \pm 2^\circ\text{C}$ ), and were subjected to continuous illumination by "day light" fluorescent tubes (80 to 100 lumen/sq foot). Each experiment contained two randomised blocks and each block was confined to one shelf in the cold room and contained 73 Petri dishes: the original dikaryotic isolate and 72 progeny. All dishes were opened every day at a fixed hour, and the first appearance of gills was recorded in every case. In a preliminary experiment, three different stages in the development of the fruit body were scored, that is the opening of the first fruit body, the initiation of sporulation and the appearance of the first gill. These three stages were found to follow each other quite closely (within 2 to 3 days), and therefore the time from inoculation to the first appearance of the gills—which was the easiest to define and to score—was chosen; it is referred to henceforth as "fruiting time." Each experiment lasted 30 days. The dikaryons which had not fruited in this time were given a score of 31, and the statistical analysis was suitably adjusted when both duplicates of the same dikaryon were given this score.

*Fruit weight* was measured on the same fruit bodies used previously for recordings of fruiting time. On the 30th day from inoculation, the fruit bodies were removed from the vegetative mycelium, and their wet weight was determined (in mg). This included fruit bodies in all stages of development, fruit-like structures, and abnormal fruit body tissues.

#### RESULTS AND ANALYSES

Table 1 contains the means and standard errors of each of the duplicate  $6 \times 6$  dikaryotic combinations of progeny, together with the scores of the original wild isolates which were grown in the same experiments. Of the three characters, only

TABLE 1

*Means of parental isolates and dikaryotic progeny (each row is obtained from a separate experiment)*

	Parental dikaryons	Progeny dikaryons	
		(a-f) × (g-l)	(m-r) × (s-z)
<b>Growth rate (mm per ten days)</b>			
Isolate 1	74.25	76.1250 ± 1.4185	73.9583 ± 1.3436
Isolate 2	85.50	89.4931 ± 1.1314	83.0000 ± 1.2933
Isolate 3	77.50	69.3958 ± 2.0340	75.5556 ± 2.2476
Isolate 5	62.00	52.1597 ± 2.2158	53.7083 ± 1.7804
Isolate 6	75.75	67.4653 ± 3.2067	70.1319 ± 2.0015
<b>Fruiting time (days from inoculation)</b>			
Isolate 1	16.50	13.8472 ± 1.1790	17.7222 ± 1.8166
Isolate 2	10.00	14.5000 ± 2.0934	18.0000 ± 2.2473
Isolate 3	5.50	6.8611 ± 0.5230	8.5139 ± 1.1730
Isolate 4	5.00	4.6944 ± 0.7382	6.1250 ± 1.6016
Isolate 5	4.00	6.2500 ± 0.6641	8.5555 ± 1.0898
Isolate 6	7.50	5.7500 ± 0.9568	6.7361 ± 0.7688
<b>Fruit weight (g)</b>			
Isolate 1	.1480	.65899 ± .06499	.66518 ± .08239
Isolate 5	.8460	.72871 ± .06173	.70772 ± .07036
Isolate 6	.8420	.90590 ± .05482	.87796 ± .05339

fruiting time was measured on progenies of all six isolates, variation for growth rate and fruit weight being determined on progenies of five and three isolates, respectively. The statistical and biometrical analyses of the original data followed closely the examples given in detail by SIMCHEN and JINKS (1964), which are also included in the present survey. The general features of the analyses are as follows. The analysis of variance provides estimates of the environmental ( $V_E$ ), heritable additive ( $V_p$ ) and heritable nonadditive ( $V_I$ ) components of variation. The nature of the nonadditive heritable component can be examined by calculating the arrays' variances ( $V_r$ ) and covariances ( $W_r'$ ). When the  $W_r'/V_r$  graphs show a significant slope of  $\frac{1}{2}$ , a model assuming additivity and dominance will explain sufficiently the variation found. The  $W_r'/V_r$  graph, however, is sensitive to gene frequencies, and the slope will be  $\frac{1}{2}$  only when the frequencies of both alleles are equal for all loci ( $u = v = 0.5$ ), as we expect for the progeny of a single dikaryon. When  $u$  is different from 0.5 but equal for all loci, a slope different from  $\frac{1}{2}$  but significantly different from 0, will result. Because of the small sample size (six monokaryons each) we might expect the  $W_r'/V_r$  slope to be occasionally disturbed due to chance deviations from  $u = v = 0.5$ . One expects such unbalanced samples to affect only their own  $W_r'/V_r$  slopes and not the slopes of the other samples of progeny with which they are crossed.

*Growth rate:* Table 2 summarizes the analyses of variance for dikaryotic growth rates of progenies of five isolates, and Figure 1 contains the estimates of the components of variation derived from these analyses. As pointed out earlier, the two sets of progeny of each isolate provide us with duplicate genetic samples,

TABLE 2

Mean squares from the analyses of variance for dikaryotic growth rate of progenies of five wild isolates

	df	Isolate 1	Isolate 2	Isolate 3	Isolate 5	Isolate 6
Dikaryons:						
Common parents ( <i>a-f</i> )	5	173.92***	512.12***	327.66**	2246.77***	119.69*
Common parents ( <i>g-l</i> )	5	131.35***	118.82	110.56	1067.41***	262.29***
Parents' interaction	25	16.14**	63.86***	91.44***	132.79***	42.79
Blocks	3	70.19***	45.45***	136.63**	32.92	336.93***
Blocks × Dikaryons (error)	105	8.05(2)	5.12(1)	22.08	19.64	40.96(1)
Dikaryons:						
Common parents ( <i>m-r</i> )	5	94.72**	1051.88***	349.65***	2967.88***	652.29***
Common parents ( <i>s-z</i> )	5	97.43***	279.45***	643.56***	1305.67***	279.16*
Parents' interaction	25	16.23**	27.27***	46.75*	88.36***	85.86***
Blocks	3	121.21***	71.15***	11.62	9.19	684.08***
Blocks × Dikaryons (error)	105	7.21(3)	6.69(1)	16.55(1)	12.68	16.02(1)

Significance levels: \* 0.05-0.01, \*\* 0.01-0.001, \*\*\* <0.001.  
The numbers in brackets ( ) designate the number of degrees of freedom lost as a result of missing tubes.

and therefore the agreement between the estimates obtained from these two sets provides a yardstick to the reliability of the sampling procedures. The duplicate estimates presented in Figure 1 agree with one another fairly well in most cases. The only exception is isolate 6 of which the two samples of dikaryotic progeny

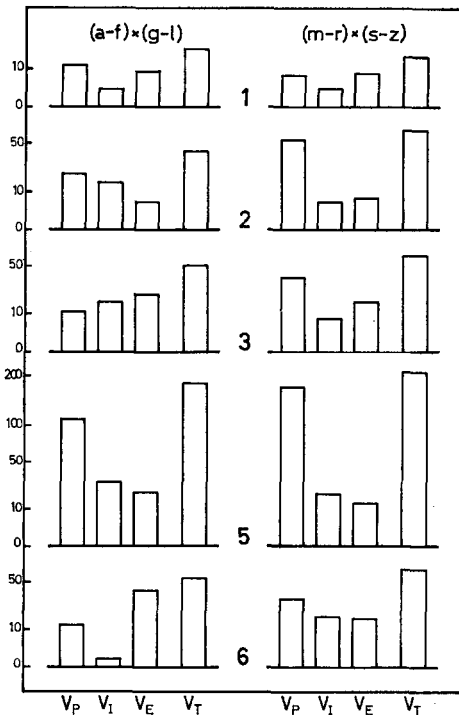


FIGURE 1.—Estimated components of variation of dikaryotic growth rate  $V_T = V_P + V_I + V_E$  (see the text for explanation). Note that the scale is not linear but adapted to the "squared" nature of the variances.

give completely different partitions of the total variation  $V_T$ . It should however be emphasized that the estimates of the components of variation which are obtained from the analysis of variance are related to one another, and are subjected to standard errors of their own.

Comparisons between estimates obtained from different isolates disclose, on the other hand, two significant differences between the various isolates.

(i) There are marked differences in the magnitude of the total variation found among the dikaryotic progeny ( $V_T = V_P + V_I + V_E$ ). This means that different degrees of heterozygosity at loci controlling growth rate existed in the original wild dikaryons (or more correctly, in the diploid nuclei of the isolates' basidia), and therefore the variation produced by the segregation of these loci among the progenies differs accordingly. Thus the highest degree of heterozygosity was shown by isolate 5, while isolate 1 showed the lowest degree of heterozygosity for loci controlling dikaryotic growth.

(ii) The two English isolates—1 and 2—gave  $V_E$  estimates consistently lower than those obtained from the three American isolates. It was also noticed that among the progenies of the latter, and in particular among the progeny of isolate 5, the instability of rate of growth over replicates was confined to certain dikaryotic combinations. Bartlett tests of homogeneity (SNEDECOR 1956, p. 285) were therefore applied to the variances of the individual combinations over blocks (Table 3), which showed the variances among five of the six groups of progeny not to be homogeneous. This result supported the belief that the mycelial instability is genetically determined. When, however, an analysis of variance was

TABLE 3

*Bartlett tests of homogeneity of variation between replicates*

	$(a-f) \times (g-l)$		$(m-r) \times (s-z)$	
	$\chi^2_{[385]}$	P	$\chi^2_{[385]}$	P
Isolate 3	53.3	0.05-0.02	81.5	<0.001
Isolate 5	146.6	<0.001	93.1	<0.001
Isolate 6	75.0	<0.001	45.7	~0.10

TABLE 4

*Mean squares of the analyses of variance of the logarithms of variances between replicates*

	df	Isolate 3	Isolate 5	Isolate 6
Common parents ( $a-f$ )	5	0.2390	0.5452	0.3548
Common parents ( $g-l$ )	5	0.2028	1.2600*	0.2800
Interaction	25	0.2120	0.3970	0.2209
Common parents ( $m-r$ )	5	0.5229	0.1888	0.3967
Common parents ( $s-z$ )	5	0.2847	0.3324	0.1628
Interaction	25	0.3377	0.2039	0.1530

\*  $0.01 < P < 0.05$ .

applied to the logarithms of these variances (Table 4), the common parents' mean squares were not found to be significantly greater than the interactions' mean squares. Thus additivity of genes controlling this instability could not be demonstrated. The high environmental variance is therefore the property of specific *genic combinations* rather than a general property of the dikaryotic derivatives of particular parental monokaryons.

The  $W_r'/V_r$  slopes, which were calculated to test the model assuming dominance as the only type of interaction contributing to the  $V_I$  estimates, are given in Table 5. The adequacy of dominance was confirmed in three of the five isolates. It appears that a similar situation exists also for the other two isolates (3 and 6), but sampling errors—which are reflected in the poor agreement between the “duplicate” estimates (Figure 1)—and high  $V_E$  values, have upset the relation between the variances and the covariances among the progenies of these two isolates.

*Fruiting time:* The progenies of all six isolates were grown in six separate fruiting experiments, and the time of fruiting was recorded for each dikaryotic combination. The results of the analyses of variance and the  $W_r'/V_r$  computations are given in Tables 6 and 7. The estimates of the components of variation are given in Figure 2.

Of the six isolates, only isolate 6 gave a consistent picture in the two independent duplicate  $6 \times 6$  groups of dikaryotic progeny. Moreover, it was the only isolate for which the  $W_r'/V_r$  graphs behaved as expected on the assumption that dominance is the only interaction between the parental haploid genotypes. Of the remainder, isolates 1 and 5 gave fairly consistent estimates of the components of variation, while the independent duplicate samples of progeny from isolates 2, 3, and 4 gave markedly different results. Dominance alone, however, cannot adequately account for the nonadditive heritable component ( $V_I$ ) of any of these five isolates.

There appears to be a difference between the mean fruiting time of the two

TABLE 5

*Regression coefficients (b) of  $W_r'/V_r$  graphs for dikaryotic growth rates*

	(a-f)	(g-l)	(m-r)	(s-z)	Dominance
Isolate 1	0.4238 ± 0.0513 **	0.3859 ± 0.0808 **	0.5205 ± 0.1218 *	0.2930 ± 0.1674	sufficient
Isolate 2	0.0931 ± 0.1519	0.4652 ± 0.0562 **	0.5334 ± 0.0282 **	0.5472 ± 0.0438 **	sufficient
Isolate 3	0.2224 ± 0.1492	0.3432 ± 0.1213 *	0.3597 ± 0.0952 *	0.2721 ± 0.2828	almost sufficient
Isolate 5	0.4123 ± 0.0599 **	0.6593 ± 0.0825 **	0.3300 ± 0.0436 **	0.4848 ± 0.0571 **	sufficient
Isolate 6	0.3622 ± 0.0490 **	0.3382 ± 0.2526 †	0.5475 ± 0.1572 *	0.3740 ± 0.1618	almost sufficient

\*\* b significantly different from 0 ( $P < 0.01$ ).

\* b significantly different from 0 ( $0.01 < P < 0.05$ ).

† b significantly different from  $1/2$  ( $0.01 < P < 0.05$ ).

TABLE 6  
*Mean squares from the analyses of variance for fruiting time*

	df	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Dikaryons: Common parents ( <i>a-f</i> )	5	92.61**	84.43***	326.42**	4.46**	13.67**	36.80***
Common parents ( <i>g-l</i> )	5	132.41***	53.93**	58.99	19.72***	9.30*	42.60***
Parents interaction	25	19.31***	13.17	64.86***	1.02	3.51***	6.04***
Blocks	1	0.31	0.22	0.50	0.89	0.12	0.22
Blocks × Dikaryons (error)	35	2.78	8.77	0.55 (3)	1.09	0.88	1.85
Dikaryons: Common parents ( <i>m-r</i> )	5	97.39**	716.13***	1001.18***	12.96	12.46*	61.78***
Common parents ( <i>s-z</i> )	5	42.02	255.43*	5.18	147.09***	44.19***	29.52***
Parents' interaction	25	17.86**	96.37***	6.87**	21.81***	5.47**	6.72***
Blocks	1	0.88	16.06	1.68	19.01	0.89	0.14
Blocks × Dikaryons (error)	35	6.60	10.06	2.75 (2)	5.13	2.38	1.18

Significance levels: \* 0.05-0.01, \*\* 0.01-0.001, \*\*\* <0.001.

TABLE 7

Regression coefficients (*b*) of  $W'_r/V_r$  graphs for fruiting time

	(a-f)	(g-l)	(m-r)	(s-z)	Dominance
Isolate 1	0.2732 ± 0.0631 * †	0.2389 ± 0.0675 * †	0.3201 ± 0.0689 **	0.2866 ± 0.0727 * †	not sufficient
Isolate 2	0.1810 ± 0.1004 †	0.2974 ± 0.1324	0.1839 ± 0.1323	0.3390 ± 0.0316 ** ††	not sufficient
Isolate 3	0.1599 ± 0.0034 ** ††	0.4994 ± 0.0145 **	0.0976 ± 0.0237 * ††	0.5854 ± 0.0364 **	not sufficient
Isolate 4	0.5354 ± 0.1698 *	0.3031 ± 0.0527 ** †	0.3087 ± 0.0551 ** †	0.1453 ± 0.0168 ** ††	not sufficient
Isolate 5	0.2147 ± 0.0720 * †	0.1746 ± 0.1092	0.4775 ± 0.0640 **	0.2241 ± 0.1059	not sufficient
Isolate 6	0.3518 ± 0.0731 **	0.3775 ± 0.1114 *	0.1104 ± 0.1253 †	0.4695 ± 0.0820 **	sufficient

\*\* *b* significantly different from 0 ( $P < 0.01$ ).  
 \* *b* significantly different from 0 ( $0.01 < P < 0.05$ ).  
 †† *b* significantly different from  $\frac{1}{2}$  ( $P < 0.01$ ).  
 † *b* significantly different from  $\frac{1}{2}$  ( $0.01 < P < 0.05$ ).

English isolates and their progeny, and the fruiting time of the four American isolates and their progeny (Table 1), the latter being much the faster fruiterers in our experiments.

*Fruit weight:* The analysis of variance of the weight measurements, the components of variation estimated from the analyses, and the  $W'_r/V_r$  are given in

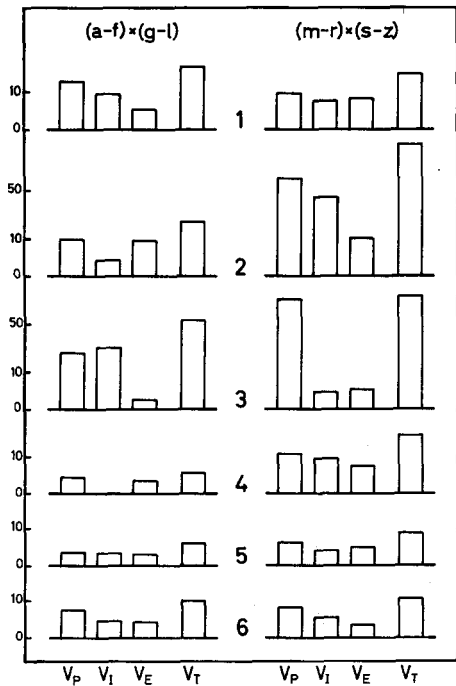


FIGURE 2.—Estimated components of variation of fruiting time.



TABLE 8

*Mean squares from the analyses of variance for fruit weight*

	df	Isolate 1	Isolate 5	Isolate 6
Dikaryons: Common-parents ( <i>a-f</i> )	5	.1803***	.1102	.0520*
Common parents ( <i>g-l</i> )	5	.0288	.2006*	.0897**
Parents interaction	25	.0276***	.0523***	.0198***
Blocks	1	.0080	.0008	.0281
Blocks × Dikaryons (error)	35	.0084	.0076	.0060
Dikaryons: Common parents ( <i>m-r</i> )	5	.2143**	.0663	.0667**
Common parents ( <i>s-z</i> )	5	.0869	.1834*	.0420*
Parents interaction	25	.0400**	.0636***	.0131*
Blocks	1	.0248	.0385	.0197
Blocks × Dikaryons (error)	35	.0136	.0099	.0057

Significance levels: \* 0.05-0.01, \*\* 0.01-0.001, \*\*\* &lt; 0.001.

TABLE 9

*Regression coefficients (b) of  $W'_r/V_r$  graphs for fruit weight*

	( <i>a-f</i> )	( <i>g-l</i> )	( <i>m-r</i> )	( <i>s-z</i> )	Dominance
Isolate 1	0.0496 ± 0.0708 ‡‡	0.2365 ± 0.0598 *	0.2775 ± 0.1236	0.2769 ± 0.0593 **	not sufficient ‡
Isolate 5	0.7883 ± 0.0685 ** †	-0.0508 ± 0.1196 ‡‡	0.4528 ± 0.2446	0.2005 ± 0.0461 * ‡‡	not sufficient
Isolate 6	0.3874 ± 0.0576 **	0.3835 ± 0.0886 *	0.2268 ± 0.0516 * †‡	0.3527 ± 0.1143 *	sufficient

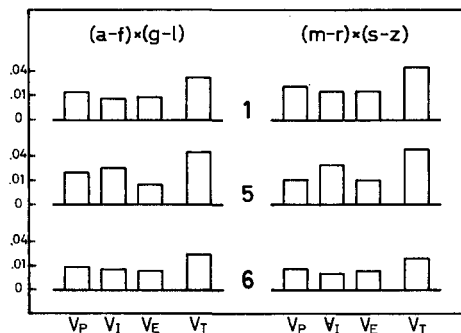
\*\* *b* significantly different from 0 ( $P < 0.01$ ).\* *b* significantly different from 0 ( $0.01 < P < 0.05$ ).‡‡ *b* significantly different from  $1/2$  ( $P < 0.01$ ).†‡ *b* significantly different from  $1/2$  ( $0.01 < P < 0.05$ ).

FIGURE 3.—Estimated components of variation of fruit weight.

Table 8, Figure 3 and Table 9, respectively. Results which were consistent over the independent duplicate samples were obtained from the progeny of isolate 6, as indeed was the case with the results for fruiting time which were described earlier. Similarly, the  $W'_r/V_r$  graphs of the progeny of isolate 6 agreed with that

expected on a model assuming a simple genetical control based on additivity and dominance only.

Thus nonallelic interactions between genetic factors controlling the characteristics of fruiting are apparently present in five of the six wild isolates. Isolate 6 differs from the other five in its simple genetic make-up for both fruiting time and fruit weight.

*Monokaryotic fruiting:* One further set of observations will be mentioned here, although not directly related to the genetic control of dikaryotic characters. It was noticed that several of the monokaryotic progeny of isolate 5 fruited readily when kept in culture for long periods. The fruit bodies produced were irregular in shape, and released only a relatively low number of basidiospores.

RAPER and KRONGELB (1958), investigating monokaryotic fruiting in *Schizophyllum*, found it among progenies of four dikaryotic stocks that were maintained in culture for long periods of time, and in one recently collected stock, out of a total of 58 stocks examined. Other Hymenomycetes in which monokaryotic as well as dikaryotic fruiting have been reported are *Collybia velutipes* (ZATTLER 1924; BRODIE 1936), *Coprinus lagopus* (HANNA 1928), *Peniophora ludoviciana* (BIGGS 1938), and *Lenzites trabea* (BARNETT and LILLY 1949). For the latter fungus it was suggested that the same genetic factors might be responsible for monokaryotic and dikaryotic fruiting. RAPER and KRONGELB (1958), however, concluded from their own experiments with *S. commune* that "There is obviously no necessary correlation between good haploid and good dikaryotic fruiting. . . . This lack of correlation would indicate different genetic bases for fruiting under the two different circumstances."

The 24 monokaryotic progeny of isolate 5 were grown in a fruiting experiment separated from, but under the same conditions, as their dikaryotic derivatives. Twelve of the monokaryons fruited in both duplicates within 22 days from inoculation, and one monokaryon fruited only in one duplicate. No correlation could be demonstrated between the ability of a monokaryotic parent to fruit or its fruiting time, and the fruiting time of the dikaryotic combinations.

*The growth rate relationships between dikaryons and their component monokaryons:* It has already been shown that growth rates of monokaryons and dikaryons are only partially correlated (SIMCHEN and JINKS 1964; SIMCHEN 1965), which indicates that many of the genes which determine the growth of dikaryons do not act in the monokaryotic stage. It is a commonly expressed opinion, on the other hand, that dikaryons grow always faster than monokaryons since, like heterokaryons in the Ascomycetes, they are maintained by virtue of their vegetative superiority over their monokaryotic components. We have already suggested (SIMCHEN and JINKS 1964) that the relation between dikaryons and their component monokaryons is genetically determined and can vary from one species to another. We have also shown that among the dikaryotic progeny of isolates 1 and 2 of *S. commune*, some grew faster than both parental monokaryons, others grew at a rate which was intermediate between the two monokaryotic growth rates, and a few dikaryons grew slower than either parental monokaryons; but the dikaryon was always stable and did not break down into

TABLE 10

*The relationship between the growth rates of dikaryons and parental monokaryons among progeny of single wild isolates*

Original isolate and monokaryotic parents	(i) Dikaryon faster than monokaryons	(ii) Dikaryon intermediate	(iii) Dikaryon slower than monokaryons
1. $(a-f) \times (g-l)$	17	18	1
$(m-r) \times (s-z)$	26	9	1
2. $(a-f) \times (g-l)$	32	4	0
$(m-r) \times (s-z)$	27	8	1
3. $(a-f) \times (g-l)$	15	12	9
$(m-r) \times (s-z)$	9	19	8
5. $(a-f) \times (g-l)$	1	11	24
$(m-r) \times (s-z)$	4	9	23
6. $(a-f) \times (g-l)$	8	7	21
$(m-r) \times (s-z)$	11	19	6

Category (ii) includes all the dikaryons that do not exceed the faster parental monokaryon and do not fall short of the slower parent.

its monokaryotic components even in the latter cases. Even more extreme situations could be found among the progeny of isolate 5, where some dikaryons had less than half the growth rate of their parental monokaryons. Table 10 shows the relationships between the growth rates of dikaryons and parental monokaryons, which were obtained from the same growth tube experiments which were reported earlier, for progenies of five wild isolates. As the two  $6 \times 6$  sets of crosses were in each case duplicate samples of progeny, the relationships between dikaryons and monokaryons are also genuinely duplicated for each wild isolate. The duplicate samples agree with one another fairly well for all wild isolates except isolate 6, for which a contingency chi-square was found to be highly significant,  $\chi^2_{[2]} = 14.3$ ,  $P < 0.001$  (for isolate 1 the chi-square was of borderline significance,  $\chi^2_{[1]} = 4.7$ ,  $P = 0.05-0.02$ ).

The relationships as they are summarized in Table 10 differ considerably between the different isolates. A contingency chi-square was therefore performed on the four isolates whose duplicate samples did not differ (1,2,3 and 5), after pooling the two duplicates for each isolate. This comparison gave  $\chi^2_{[6]} = 141.8$ ,  $P < 0.001$ . Thus the relationships between dikaryons and parental monokaryons are unique for each isolate, which strongly supports our previous suggestion that they are genetically determined.

*Correlations between dikaryotic characters:* The experiments reported above could also provide us with information concerning the correlations between different characters, since the measurements were taken from the same sets of dikaryotic combinations. Such correlations could either arise by linkage between heterozygous loci affecting independently the correlated characters, or by pleiotropic effects of the same genes on two or more characters. Correlations resulting from linkage can vary according to whether the linked genes on the parental chromosomes are in the coupling or repulsion phases, and whether they are

TABLE 11  
*Correlations between characters (r values for 34 df) calculated from  
the means of the dikaryotic combinations*

	Growth rate- fruiting time	Growth rate- fruit weight	Fruiting time- fruit weight
No. 1. (a-f) × (g-l)	-0.0202	0.2492	-0.6120***
(m-r) × (s-z)	-0.1389	0.0500	-0.1295
No. 2. (a-f) × (g-l)	-0.1243	.....	.....
(m-r) × (s-z)	-0.1500	.....	.....
No. 3. (s-f) × (g-l)	0.1259	.....	.....
(m-r) × (s-z)	0.0782	.....	.....
No. 5. (a-f) × (g-l)	0.0350	-0.2253	0.1594
(m-r) × (s-z)	-0.1597	0.3560*	-0.4628**
No. 6. (a-f) × (g-l)	0.2825	-0.2987	0.0742
(m-r) × (s-z)	0.2697	-0.1359	0.0202

\*\*\* P < 0.001    \*\* 0.001 < P < 0.01    \* 0.02 < P < 0.05.

heterozygous or homozygous. Pleiotropic correlations, on the other hand, would not be expected to differ so much from one situation to another.

Table 11 contains 22 correlation coefficients, only three of which are significant. It is not possible therefore to postulate genetical correlations between any of the dikaryotic characters which were determined during the course of the experiments reported here.

#### DISCUSSION

That natural variation exists for characters such as fruiting or growth is not a new fact. The existence of such variation has already been demonstrated for *S. commune* itself (RAPER and KRONGELB 1958). However, in the present study we have tried to investigate the nature of the polygenic system controlling this variation by the use of suitable biometrical methods.

The polygenic system controlling growth rate of dikaryotic mycelia has proved to be a simple system consisting of genes with additive and dominance effects only. The two fruiting characters, on the other hand, behaved in a more complicated and unpredictable way, and the nonadditive heritable variation disclosed for these characters could not be explained by dominance alone. Interaction mechanisms other than dominance must have been involved in the fruiting processes, at least among the progenies of isolates 1 to 5. These two characters—fruiting time and fruit weight—are also more complex than growth rate on the level of phenotypic expression. While every cell in the fungal colony takes part, at some time or another, in growth (which is elongation and division of cells), only a few cells give rise to the fruit bodies. Even if the whole mycelium is developing towards fruiting (and this has not yet been proved satisfactorily) the fruit bodies themselves are initiated in single dikaryotic cells (BULLER 1931; KNIEP 1930), although these themselves are probably stimulated towards fruiting by their neighbouring cells (WESSELS 1965).

Another problem of interest is whether dominance for a particular character is unidirectional or ambidirectional, since such information suggests the type of natural selection to which the character has been subjected (MATHER 1960, 1966). In our experiments, we can learn about the relative degrees of dominance of the genotypes involved in the crosses from their order on the  $W_r'/V_r$  graphs: the most dominant arrays have the lowest variances and covariances (JINKS 1954; SIMCHEN and JINKS 1964). Here, only fruiting time shows definite signs of unidirectional dominance which implies that natural selection is of the directional type, and favours early fruiterers (which are the dominant genotypes). But this is not so for all sets of crosses. As we have seen earlier, the polygenic system controlling this character is also distinguished by the presence of nonallelic interactions. Such interactions, however, are also characteristic of polygenic systems which are subjected to directional selection, in contrast to systems which are subjected to stabilising selection (MATHER 1960, 1966). Dikaryotic growth rate, when it is not very low, shows ambidirectional dominance, and so does fruit weight. Thus we have evidence that fruiting time is a fitness character which is subjected to directional selection in nature, while the other two characters are probably not so closely correlated with fitness as such, and are subjected to a stabilising selection. These results are comparable to our own results with *Collybia velutipes* (SIMCHEN 1965), where dominance was found to be unidirectional for time to the appearance of the first primordium (of a fruit body), but not for the other two fruiting characters or for dikaryotic growth rate.

Each of the six wild isolates of *S. commune* gave rise to a "population" of 72 dikaryons. The marked differences between the mean performances of these "populations" and between the amounts of variation they displayed must reflect differences between the original isolates in the degrees of heterozygosity and in the relative frequencies of increasing and decreasing alleles present in the polygenic systems controlling the variation. In general, the American isolates have higher  $V_E$  components for dikaryotic growth rate than the English isolates, and this is probably genetically determined. Another significant trend is that the American isolates and their derivatives fruit faster under our experimental conditions. Whether these differences are genuine geographic differences is difficult to determine, since the sample of isolates from each area that was examined was small. The only wide-range genetical survey that has been carried out in *Schizophyllum* (RAPER, KRONGELB and BAXTER 1958) was concerned with the geographical distribution of the incompatibility factors and this failed to indicate any patterns of geographical divergence. We cannot, however, compare continuous variation of the type explored here with the variation for the incompatibility factors, since in the latter system rare factors are automatically selected for. Thus the compatibility factors of nuclei from a "foreign" environment will always be favoured, while the rest of their genomes will be selected against unless advantageous in the "local" conditions.

Another difference between isolates appears to be in the relationships between dikaryons and parental monokaryons. The extreme relationships were found among the progeny of isolate 2, where five sixths of the dikaryons grew faster

than their respective monokaryons, and among the progeny of isolate 5, where two thirds of the dikaryons grew slower than any of their parental monokaryons. It appears that this is yet another property for which the wild isolates of *S. commune* show genetical variation, in addition to those already described in this paper and elsewhere (RAPER *et al.* 1958; SIMCHEN 1966).

I am indebted to PROFESSOR J. L. JINKS for his interest and encouragement throughout and for his advice during the preparation of the manuscript. Part of this work was undertaken while in receipt of the Rosalind Franklin Grant from the Friends of the Hebrew University of Jerusalem, London N.W. 1, and it was completed while financed by the Agricultural Research Council of Great Britain.

#### SUMMARY

The variation for three dikaryotic characters has been assessed among the progenies of six wild isolates by the use of the biometrical methods described by SIMCHEN and JINKS (1964). Growth rate of dikaryotic mycelia is probably controlled by a simple polygenic system consisting of additive and dominance effects only. Genic interactions other than dominance are probably involved in the control of fruiting time and fruit weight. No correlation between any two of the three characters could be demonstrated.—There are differences between the isolates in the total amount of variation recovered from their progenies, as well as in the relative magnitudes of the components of variation. The isolates also differ in the mean performances of their dikaryotic progeny and in the relationships between dikaryotic and monokaryotic growth rates.

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