THE GENETICS OF *SORDARIA BREVICOLLIS*. II. BIASED SEGREGATION DUE TO SPINDLE OVERLAP¹

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BIASED segregation in the second meiotic division has been reported in only a few ascomycetes. Several workers (LINDEGREN and LINDEGREN 1937, 1942; WHITEHOUSE and HALDANE 1946; BOONE and KEITT 1956; BISTIS 1956) have reported a preponderance of asymmetrical (2:2:2:2) over symmetrical (2:4:2) second-division segregation (MII) asci with respect to a particular locus, but on a much smaller scale than is characteristic of *Sordaria brevicollis* Olive and Fantini (SHAW 1962; CHEN 1964a,b). The occurrence of this type of biased segregation has been found due to spindle overlap in the second meiotic division (Figure 1).

For a better understanding of the nature and cause of the unequal frequencies of the two types of postreduction asci, an extensive study of biased segregation in *S. brevicollis* was made. Evidence for genetic control of this phenomenon is also presented.

MATERIALS AND METHODS

The ascospore color mutants of the seven linkage groups were induced by ultraviolet irradiation. All mutants were derived from two compatible cultures (*A* and *a*) of the original type-material described by OLIVE and FANTINI (1961). They were maintained and crossed on Difco cornmeal-dextrose agar plus 0.1% yeast extract. Descriptions of the mutants and linkage data have been presented (CHEN 1965).

Crosses for routine analysis were made in Petri dishes and incubated at a constant temperature of 23°C. In the study of temperature effects on biased segregation, three temperatures (16°, 23°, 30°C) were employed. Data on the segregation patterns of asci in the hybrid perithecia were obtained by direct observation of ascus clusters, which mature about 12 days from the time of mycelial implantation.

EXPERIMENTAL RESULTS

In order to study biased segregation, crosses were made between ascospore color mutants and wild type of opposite compatibility. In ascomycetes with linearly arranged ascospores in asci without spindle overlap, the numbers of symmetrical and asymmetrical MII asci tend to be equal. In *S. brevicollis*, however, the relative sizes of these two groups are highly variable. Figure 1 illustrates how an excess of asymmetrical apparent MII asci is due to spindle overlap at the

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FIGURE 1.—Diagram of asci showing the effects of spindle overlap on segregation.

second meiotic division, converting first-division segregation (MI) asci into the asymmetrical MII pattern (SHAW 1962). The percentage of excess asci of the asymmetrical MII class is determined as follows (see Table 1):

$$\frac{(\text{Apparent asymmetrical MII}) - (\text{Symmetrical MII})}{\text{Total number of asci}} \times 100.$$

The proportions of excess invalid asymmetrical asci range from 0% in the crosses $g_4a \times g_4^+A$ and $g_4A \times g_4^+a$ (Figure 2) up to 77.8% in the cross $b_1A \times b_1^+a$ (Figure 3). The genetic background of the particular pair of cultures used, including unidentified genetic factors, appears chiefly responsible for the degree of biased second-division segregation observed in the different crosses. The percentage of invalid asymmetrical MII asci involving the g_2 marker is 51.6% in

TABLE 1

Crosses showing varying degrees of biased second-division segregation in S. brevicollis

Cross No.	Cross*	Locus [†]	MI	MII		Total	Percent of invalid asymmetry
				Asym-metrical	Sym-metrical		
1	$\gamma_2 a \times \gamma_2 + A$	γ_2 (I)	763	23	3	789	2.5
2	$\gamma_2 A \times \gamma_2 + a$		246	77	1	324	23.4
3	$b_1 a \times b_1 + A$	b_1 (II)	186	395	15	596	63.8
4	$b_1 A \times b_1 + a$		129	588	17	734	77.8
5	$g_3 a \times g_3 + A$	g_3 (II)	485	12	10	507	0.4
6	$g_3 A \times g_3 + a$		428	165	10	603	25.7
7	$g_4 a \times g_4 + A$	g_4 (II)	49	61	62	172	0
8	$g_4 A \times g_4 + a$		404	328	332	1,064	0
9	$g_2 a \times g_2 + A$	g_2 (III)	156	201	11	368	51.6
10	$g_2 A \times g_2 + a$		473	47	14	534	6.1
11	$g_5 a \times g_5 + A$	g_5 (IV)	1,015	493	250	1,758	13.7
12	$g_5 A \times g_5 + a$		800	211	145	1,156	5.7
13	$\gamma_4 a \times \gamma_4 + A$	γ_4 (V)	177	87	0	264	32.5
14	$\gamma_4 A \times \gamma_4 + a$		412	236	0	648	36.4
15	$h_3 a \times h_3 + A$	h_3 (VI)	506	72	31	609	6.7
16	$h_3 A \times h_3 + a$		896	173	52	1,121	10.8
17	$g_7 a \times g_7 + A$	g_7 (VII)	1,432	401	42	1,875	19.1
18	$g_7 A \times g_7 + a$		342	226	8	576	37.8

* Analysis for types of asci does not involve compatibility alleles.

† Linkage group in parentheses.

Cross 9 (Table 1), while it is only 6.1% in Cross 10, in both of which the known markers (at compatibility and g_2 loci) are the same. Similar crosses involving g_3 give very different invalid asymmetry values, Crosses 5 and 6 exhibiting 0.4% and 25.7% excess asymmetrical asci, respectively.

The effects of temperature upon segregation were studied in a single cross, $g_5 A \times g_5 + a$ (Table 2). No significant change in the amount of second division segregation or in the proportion of invalid MII asci occurred when the crosses were grown at 30°C as compared to maintenance at the usual temperature of 23°C. However, when crosses were grown at 16°C, the proportion of legitimate MII asci dropped from 25% at 23°C to 11.3% and the invalid MII asci decreased

FIGURE 2.—Cluster of hybrid asci from the cross, $g_4A \times g_4+a$, showing about equal numbers of symmetrical and asymmetrical MII asci. $\times 200$.

FIGURE 3.—Cluster of hybrid asci from the cross, $b_1A \times b_1+a$, showing a preponderance of asymmetrical MII asci. $\times 200$.

from 26.7% to 16.7%, the latter proportionately somewhat less of a decrease. The effects of variations in temperature upon second-division segregation were previously described for three spore-color loci in *Sordaria fimicola* (OLIVE 1956), in which the fluctuations in proportions of MII asci were interpreted in terms of temperature effects upon crossing over.

There was no indication of polarized segregation in MI asci for any of the loci of *S. brevicollis* studied. Thus, there is no tendency for one allele to pass towards one end of the ascus more frequently than towards the other end.

DISCUSSION

The occurrence of biased second-division segregation is an outstanding feature of *S. brevicollis*. The significantly higher percentage of apparent asymmetrical MII asci observed in this species is due to partial spindle overlap in the second

TABLE 2

Temperature effects on biased segregation for spore color in the cross $g_5A \times g_5+a$

Temperature (°C)	MI	MII		Total	Legitimate MII asci	Invalid MII asci
		Asym- metrical	Sym- metrical			
30	884	730	224	1,838	24.3%	27.5%
23	936	812	271	2,019	25.0%	26.7%
16	2,177	676	171	3,024	11.3%	16.7%

meiotic division (SHAW 1962; CHEN 1964b). The data are fully consistent with this concept. It is important to recognize the occurrence and extent of spindle overlap, since failure to do so leads to inaccurate mapping of centromere distances (CHEN 1964a, 1965). WHITEHOUSE (1942) has shown that spindle overlap at the second meiotic division converts tetrads from the MI class to the asymmetrical MII class, leaving the symmetrical MII class unaffected (Figure 1). On the other hand, similar spindle overlap converts asymmetrical MII asci to the MI class. The expected MII frequency is obtained simply by doubling the number of symmetrical MII asci, which have remained unaffected by spindle overlap, since the two classes of MII asci should normally be equal in number.

The extent of MII biased segregation varies markedly in accordance with the strains crossed. This feature has been demonstrated in crosses involving the same mutants in different combinations and in reciprocal combinations with the compatibility factors. For example, the proportion of invalid asymmetrical MII asci was 0.4% in the cross, $g_3a \times g_3^+A$, and 25.7% in the reciprocal cross, $g_3A \times g_3^+a$.

Approximately equal numbers of asymmetrical and symmetrical MII asci were obtained in matings between the g_i mutant and wild type in reciprocal crosses, $g_4a \times g_4^+A$ and $g_4A \times g_4^+a$. These are the only such crosses in which no invalid asymmetrical asci were indicated. A lack of biased segregation in these crosses could be due primarily to the genetic makeup of the pairs of parental cultures, or even to the suppression of spindle overlap by the mutant g_i locus. However, since the g_4 mutant shows 62.5% MII segregation and therefore suggests a position of about 31.3 crossover units from the centromere of linkage group II (CHEN 1964a, 1965), this distant location is more likely to explain the approximately equal numbers of asymmetrical and symmetrical MII asci. If a marker is 33.3 units or more from the centromere, thus giving a valid second-division segregation of 66.7%, the three classes of asci—MI, MII symmetrical, and MII asymmetrical—should normally be equal in number. In such cases, spindle overlap will equally affect the conversion of MI into MII asymmetrical and MII asymmetrical into MI asci, thus establishing an equilibrium between the two and leaving both of the MII classes equal in number, the MII symmetrical class being unaffected by spindle overlap. Further crossing tests, employing simultaneously an additional marker nearer the centromere, should clarify this problem.

With regard to the simultaneous biased segregation for two linked or unlinked loci close to the centromere, a similar degree of biased segregation is observed for both in the same cross, since both are affected equally by the occurrence of spindle overlap, as data in Table 3 show.

Genetic studies of *S. brevicollis* are being continued in our laboratory.

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SUMMARY

Biased segregation, traceable to spindle overlap in the second meiotic division, has proved to be a common feature of *Sordaria brevicollis*. The extent of biased segregation (0 to 78%) is probably affected by a number of unidentified genetic

TABLE 3

Data on the simultaneous biased segregation for two linked or unlinked loci near centromeres

Cross*	Locus†	MI	MII		Total	Percent of invalid asymmetry
			Asymmetrical	Symmetrical		
$g_3b_1+a \times g_3+b_1A$	g_3 (II)	282	269	11	562	45.9
	b_1 (II)	283	272	7	562	47.2
$g_3g_2+a \times g_3+g_2A$	g_3 (II)	329	108	16	453	20.2
	g_2 (III)	332	111	10	453	22.2
$g_3\gamma_4+a \times g_3+\gamma_4A$	g_3 (II)	468	89	10	567	13.9
	γ_4 (V)	476	91	0	567	13.8
$b_1\gamma_4+A \times b_1+\gamma_4a$	b_1 (II)	101	545	16	662	79.9
	γ_4 (V)	102	541	19	662	78.8
$g_3\gamma_4+A \times g_2+\gamma_4a$	g_2 (III)	266	227	12	505	42.5
	γ_4 (V)	262	237	6	505	45.7

* Analysis for types of asci does not involve compatibility alleles.

† Linkage group in parentheses.

factors. The greater frequency of asymmetrical (2:2:2:2) over symmetrical (2:4:2) "postreduction" asci that is typical of *S. brevicollis* is discussed in terms of the occurrence of spindle overlap in the second meiotic division.

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