Genet. Res., Camb. (1967), **10**, *pp.* 289–294
Printed in Great Britain

Unbiased second-division segregation in *Neurospora*

By ADRIAN M. SRB and HOWARD JAROLMEN

Section of Genetics, Development & Physiology, Cornell University, Ithaca, New York, U.S.A.

(*Received* 3 *August* 1967)

1. INTRODUCTION

Whitehouse & Haldane (1946) have examined evidence for biased segregation in the second meiotic division of *Neurospora sitophila* and *Bombardia lunata*. Data examined by them showed that for several loci, including the mating type locus, asymmetrical (2:2:2:2) 'post-reduction' asci were more frequent than the symmetrical (2:4:2) type. Biases of this kind did not appear in data obtained from *N. crassa*.

Recently we have made crosses of a large number of Neurosporas of different origin, including several distinct strains of *N. sitophila*. The results have enabled us to re-examine the question of whether certain wild-type Neurosporas other than *N. crassa* show biases in the two types of second-division segregation. The data from our material provide little if any evidence for such biases.

2. MATERIALS AND METHODS

(i) *Media and techniques*

Crosses were made on 1·7 % Difco corn meal agar in 16 × 150-mm. culture tubes and incubated at 25C. Since some of the strains do not form protoperithecia, all crosses were made by inoculating the parent strains side by side on the agar slant. Spores were isolated in order from asci by the usual technique. The processes of isolation of spores and recording of data provided for identification of the proximal and distal spores of each ascus. The experiments giving rise to the data summarized in Tables 1 and 2 were planned on the basis that asci would be isolated until 100 complete tetrads were recovered for each cross. This goal was achieved for the crosses summarized in Table 1. For certain of the crosses recorded in Table 2 the data are based on smaller samples because of problems with fertility, and in some instances the desired crosses could not be made. In the analysis of data, Yates' correction for continuity was employed in all determinations of chi-square.

(ii) *Strains of* Neurospora

The *peak* (allelic with *biscuit*) colonial mutant of *N. crassa* has been described by Murray & Srb (1962). The particular *peak* allele used in the studies reported here was *pk*-2. The various wild-type strains represent quite diverse material, as evidenced

Table 1. *Symmetrical vs. asymmetrical second-division segregations on the genetic background of* Neurospora crassa

Source of wild allele of *peak*	Peak locus		Mating type locus	
	Observed	χ^2	Observed	χ^2
N. crassa	22 (sym.)*		18	
	29 (asym.)	0·706	15	0·122
N. crassa	30		11	
	29	0	16	0·592
N. sitophila	22		5	
	26	0·188	3	0·126
N. sitophila E	29		14	
	31	0·016	15	0
N. sitophila NA	26		11	
	24	0·02	9	0·05
N. intermedia	20		10	
	29	1·306	10	0
N. tetrasperma	27		12	
	20	0·766	10	0·046
Costa Rica	20		6	
	27	0·766	10	0·562
Liberia	32		13	
	27	0·272	14	0
Philippine Islands	30		17	
	23	0·68	14	0·13
Taiwan I	29		10	
	18	2·128	10	0
Taiwan II	33		8	
	31	0·016	3	1·454
Singapore	24		6	
	32	0·876	3	0·444
Singapore K	15		10	
	30	4·356†	8	0·056
Puerto Rico	36		8	
	28	0·766	7	0
Honduras	24		10	
	28	0·174	11	0
Java	23		11	
	24	0	10	0
Java K	24		2	
	28	0·174	13	6·666‡
CPP	24		10	
	30	0·462	14	0·376
Nigeria	27		15	
	28	0	11	0·346

* For each pair under *Observed* the first figure is the number of symmetrical, the second the number of asymmetrical second-division asci.

† p is between 0·01 and 0·05.

‡ p is less than 0·01.

https://doi.org/10.1017/S0016672300011046 Published online by Cambridge University Press

Table 2. *Symmetrical vs. asymmetrical second-division segregations on various genetic backgrounds in the genus* Neurospora

	Peak locus		Mating type locus	
Genetic background	Observed	χ^2	Observed	χ^2
N. crassa	33 (sym.)*		7	
	28 (asym.)	0·262	10	0·236
N. sitophila	26		22	
	30	0·16	21	0
N. sitophila E	44		19	
	27	3·606	24	0·372
N. sitophila NA	34		37	
	31	0·062	25	1·952
N. intermedia	27		23	
	35	0·79	28	0·314
Costa Rica	16		9	
	30	3·674	11	0·05
Liberia	25		15	
	27	0·02	30	4·356†
Philippine Islands	31		10	
	43	1·636	14	0·376
Taiwan I	26		18	
	25	0	29	2·128
Taiwan II	34		18	
	26	0·816	9	2·370
Singapore	6		6	
	4	0·10	4	0·10
Singapore K	33		11	
	29	0·146	11	0
Puerto Rico	30		24	
	35	0·246	24	0
Honduras	32		24	
	40	0·68	22	0·022
Java	2		2	
	5	0·572	4	0·166
CPP	37		22	
	32	0·232	19	0·098
Nigeria	32		22	
	28	0·150	13	1·828

* For each data pair under *Observed* the first figure is the number of symmetrical, the second the number of asymmetrical second-division asci.

† p is between 0·01 and 0·05.

by morphology, by varying degrees of compatibility when intercrosses are made, and by segregations of genetic attributes following hybridization. Many of these strains represent as yet undetermined species and are designated merely by their geographic origin. The sources and designations of the wild-types are as follows. The *N. crassa* cultures are of the St. Lawrence laboratory strain, further inbred by us. The origins of the cultures designated *N. sitophila*, *N. intermedia*, Nigeria and

Philippine Islands have already been described elsewhere (Srb, 1958). *N. sitophila E* was isolated for us by C. G. Sibley near Oxford, England; it differs from the other cultures of *N. sitophila* in our possession by crossing appreciably more readily with strains of *N. crassa*. *N. sitophila NA* was isolated in North Africa and provided to us by G. Rizet. The *N. tetrasperma* was isolated from forest soil in Borneo and provided by J. H. Warcup. Strains Costa Rica and Honduras were provided by S. Freiburg from collections of the United Fruit Company. The strains called Singapore, Liberia, and Puerto Rico were provided by N. H. Horowitz and are those in the listings of the Fungal Genetics Stock Center. Strains Taiwan I, Taiwan II, Singapore K, and Java K were collected and provided to us by R. P. Korf. The strain designated Java was provided by F. J. Ryan. Strain CPP was isolated as a contaminant on a Pelvetia protein preparation from the west coast of the United States and provided by J. K. Pollard; it shows fair fertility in crosses with strains both of *N. crassa* and *N. sitophila* but does not conform well with descriptions of any recognized species of the genus.

Several of the wild-type strains used in the experiments were provided originally in only a single mating type. These were *N. intermedia*, *N. sitophila E*, Liberia, Singapore, Puerto Rico, Java, CPP, and Nigeria. The appropriate allele for opposite mating type was in each instance provided from *N. crassa* by a minimum of ten generations of backcross.

3. RESULTS

The data with reference to segregations of the *pk* allele are of two kinds. In Table 1, *pk* is seen segregating in strains where the genetic background is *N. crassa*. The parents of each cross were derived in the following manner. Each of the wild strains was crossed separately to a strain of *N. crassa* carrying the *pk* allele. In each instance a wild-type segregant was recovered and backcrossed to the strain *N. crassa peak*. Recurrent backcross was carried out for ten generations, providing a series of strains each having a wild-type allele of *peak* of different origin but on the background of *N. crassa*. In the tenth generation, asci were isolated in order. These isolations gave the results reported. The data in Table 1, therefore, are essentially control data, with the primary controls being the first two crosses listed, where the *pk* allele is segregating against the wild-type allele from which it was derived in *N. crassa*.

Table 2 reports the segregation of *pk* against wild-type alleles of different origin, in each instance on the genetic background of the wild strain providing the wild-type allele. In each cross one parent is the particular wild strain that is designated; the other is a strain obtained by ten generations of recurrent backcross of *peak* to that wild-type. Again, asci isolated in order in generation ten provide the data.

For all crosses, segregations were determined for mating type as well as for *peak*. In *N. crassa*, *pk* is in linkage group 5, mating type in linkage group 1. In none of the crosses, irrespective of the wild-types involved, did *pk* show linkage with mating type. Therefore, the results give tests for two different chromosomes.

In general, segregations on the genetic background of *N. crassa* (Table 1) give little evidence for excess either of symmetrical or asymmetrical post-reduction asci. Tests for fit to a ratio of 1 symmetrical : 1 asymmetrical give low chi-square values except for the segregation of *pk* against a wild-type allele from Singapore K and for the segregation of mating type in the cross where the wild-type allele of *pk* is derived from Java K. In Table 2, where the results sample segregations on a wide variety of genetic backgrounds, only the segregation of mating type in Liberia provides a chi-square larger than that for the 5 % level.

Chi-square tests for heterogeneity (Table 3) indicate that the data may be pooled. In the series of segregations on a genetic background of *N. crassa* pooled data for each locus fit a 1 : 1 ratio. For the *pk* locus 517 symmetrical and 542 asymmetrical asci were obtained; for the mating type locus 207 symmetrical and 206 asymmetrical asci were obtained. Likewise in the series of segregations on various wild-type

Table 3. *Heterogeneity tests for two series of crosses providing data on the numbers of symmetrical and asymmetrical second-division segregations*

Locus	Segregations on a background of *N. crassa*		Segregations on the backgrounds of various wild Neurosporas	
	χ^2	p	χ^2	p
Peak	13·128	0·80–0·90	13·114	0·50–0·70
Mating type	10·97	0·90–0·95	14·258	0·50–0·70

backgrounds, the data for the *pk* and mating type loci fit a 1 : 1 ratio. The symmetrical to asymmetrical frequencies were 468 : 475 for the *pk* locus and 289 : 298 for the mating type locus.

With data from an independent study, the mating type locus of *N. sitophila* could be tested again for a possible excess of symmetrical or asymmetrical second-division segregations. The observation was 69 symmetrical to 78 asymmetrical asci, fitting a 1 : 1 ratio with the level of p being between 0·50 and 0·70.

4. DISCUSSION

Chen & Olive (1965) have reported biased post-reduction segregations in *Sordaria brevicollis*, which they suggest to be due to spindle overlap in the second meiotic division. The data reported here show little evidence for such biases in second-division segregation asci, indicating that spindle overlap is at least rare in the *Neurospora* strains studied. The results are consistent with the cytological observations in *N. sitophila* (Wilcox, 1928) and with the implications of genetical studies in *N. crassa* by Whitehouse (1942) and others that the central nuclei formed in the second meiotic division rarely if ever pass one another.

In the studies reported here all three instances of significant deviation from an expected 1 : 1 ratio of symmetrical to asymmetrical asci indeed showed the asym-

metrical asci in excess. However, given a fairly large number of samples and small numbers in each sample, some instances of such deviations are not surprising. And it must be pointed out that in each instance the excess of asymmetrical segregations involved but one of the two loci tested in that same cross. Finally, two of the three instances of excess asymmetrical segregations occurred on the genetical background of *N. crassa*, where the evidence is that such biases are not of general occurrence. In the genus *Neurospora*, then, special and interesting instances of bias toward asymmetrical second-division segregations may well occur, but a fairly broad survey does not reveal the phenomenon as a species attribute.

SUMMARY

Results of crosses of a large number of Neurosporas of different origin, including several distinct strains of *N. sitophila*, were utilized to re-examine the question of whether certain wild-type Neurosporas other than *N. crassa* show biases in the two types of second-division segregation. Segregations for alleles of the mating type and *peak* loci on a wide variety of genetic backgrounds gave little evidence for excess either of symmetrical or asymmetrical 'post-reduction' asci.

We gratefully acknowledge technical assistance by Allene Falk and Mary Basl. Our research was supported by Grant GM 12953, National Institutes of Health, U.S. Public Health Service. One of us (H. J.) acknowledges support through a postdoctoral traineeship from the National Institute of General Medical Sciences, NIH, Grant T1 GM 1035.

REFERENCES

CHEN, K. C. & OLIVE, L. S. (1965). The genetics of *Sordaria brevicollis*. II. Biased segregation due to spindle overlap. *Genetics*, **51**, 761–766.

MURRAY, J. C. & SRB, A. M. (1962). The morphology and genetics of wild-type and seven morphological mutant strains of *Neurospora crassa*. *Can. J. Bot.* **40**, 337–349.

SRB, A. M. (1958). Some consequences of nuclear-cytoplasmic recombinations among various Neurosporas. *Cold Spring Harb. Symp. quant. Biol.* **23**, 269–277.

WHITEHOUSE, H. L. K. (1942). Crossing-over in *Neurospora*. *New Phytol.* **41**, 23–62.

WHITEHOUSE, H. L. K. & HALDANE, J. B. S. (1946). Symmetrical and asymmetrical reduction in Ascomycetes. *J. Genet.* **47**, 208–212.

WILCOX, M. S. (1928). The sexuality and arrangement of the spores in the ascus of *Neurospora sitophila*. *Mycologia*, **20**, 3–17.