INTERNUCLEAR EFFECTS ON PROTOTROPH FREQUENCIES OF SOME CROSSES IN *NEUROSPORA CRASSA*

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VARIATION in recombination frequencies has been reported in the mapping of several loci in *Neurospora crassa* (FROST 1961 and STADLER and TOWE 1962). Such variation may be a result of the diversity of the wild type ancestry of *Neurospora* stocks (LAVIGNE and FROST 1964). Variation in recombination frequency over a specific genetic region may reflect either structural differences in the chromosome over that region, or the activity of a gene or genes of unknown location.

Attempts to identify loci associated with the control of recombination using standard mapping techniques have met with only partial success (STADLER and TOWE 1962; FROST 1962; LAVIGNE and FROST 1964). However a gene affecting intragenic prototroph frequency at the *his-1* locus has been identified by JESSOP and CATCHESIDE (1965).

The present work attempts to distinguish between structural effects and genic effects in the following way: strains known to differ in recombination frequency over a specific region when crossed to a common conidial parent, may be combined in a heterokaryon. The heterokaryon may then be crossed to the same conidial parent. If the nuclear components of the heterokaryon are identifiable through the presence of appropriate marker loci, then the recombination behaviour of the component strains inside the heterokaryon may be compared with their behaviour when they are used independently. If the recombination frequency associated with a component nucleus of the heterokaryon differs significantly from the recombination frequency of that nucleus when present in a cross as a homokaryotic parent, it may be assumed that structural differences were not the cause, or were not the only cause, of the original diversity between the strains. The conclusion is that the variation in recombination frequency reflects the activity of gene products, and the internuclear exchange of such products in a heterokaryon. Although these gene products may not be recombination specific in their action, they may nevertheless be presumed to be under the influence of natural selection, at least in part, because of their action on recombination.

MATERIALS AND METHODS

The following markers were used: *arg-6* (29997), *al-2* (15300), *cys-2* (80702), *ylo* (Y30539y), *leu-3* (R156), and *pan-2* (Y154M64). The numbers in parentheses refer to the isolation numbers

of the mutants. Figure 1 shows their approximate positions on the linkage map (Although *ad-1* (3254) was present it was not used as a marker, and media were appropriately supplemented with adenine.)

Standard techniques were used in making crosses, isolating ascospores, and identifying biochemical mutants. These have been described elsewhere, e.g. by THRELKELD (1962).

Prototroph frequencies were estimated as follows. Mature ascospores were scraped off the walls of the cross tube and suspended in 0.1% agar. Aggregations of spores were broken up by agitation in a Vortex mixer. The spores were filtered through glass wool to obtain a stock suspension. 1.0 ml samples from the stock suspension were diluted, ($\times 10$, $\times 15$, or $\times 20$), to form several dilution suspensions. Known aliquots of the stock suspension were each added to 100 ml solutions of molten sorbose-fructose-glucose (SFG) medium (BROCKMANN and DE SERRES 1964) at 60°C, appropriately supplemented for prototroph selection. Known aliquots from the dilution suspensions were each added to a 100 ml solution of molten SFG complete medium. The spore suspensions in SFG medium were heat shocked at 60°C for 40 minutes, and then poured into empty petri dishes. The dishes were incubated at 37°C to avoid the leaky growth associated with the strains at 25°C.

Prototrophs were scored with the naked eye after 72 (± 2) hours, after which time the prototroph counts remained quite constant; samples of prototrophs were isolated microscopically immediately following the time of scoring. Colonies on complete medium were scored after three days and after five days, and samples were isolated after the second scoring.

Significant differences in the data were examined through the use of the chi-square test, contingency tables, and Student's t-test for paired data. The latter was used in order to detect possible significant differences between prototroph frequencies in the crosses of homokaryons and crosses involving a heterokaryon.

Thus the experiments were designed to compare the prototroph frequencies from crosses fundamentally of the following types:

$$\begin{aligned} & (cys\ pan^+)_{1\ } \text{♀} \times cys^+ \ pan\ \text{♂} \\ & (cys\ pan^+)_{2\ } \text{♀} \times cys^+ \ pan\ \text{♂} \\ & ((cys\ pan^+)_{1\ } + (cys\ pan^+)_{2\ }) \text{♀} \times cys^+ \ pan\ \text{♂} \end{aligned}$$

In order to associate prototrophic colonies and colonies grown on complete media with one of the component nuclei of the heterokaryon, different nuclear markers were introduced into each homokaryotic strain, the markers used were *ylo* (yellow conidia) and *al-2* (albino conidia). These colour markers also provided an indication of the establishment of a heterokaryon through complementation resulting in wild-type conidial pigmentation. In addition, heterokaryon forcing markers were used; *arg-6* with *al-2* and *leu-3* with *ylo*. Thus a number of the following two types of strains were finally synthesized: *cys-2 ylo*; *leu-3* and *cys-2*; *arg-6 al-2*. From these strains, those that were both heterokaryon compatible and resulted in crosses of high fertility were selected. The common conidial (fertilizing) parent for all crosses was *ad-1 pan-2*; *a*.

Strains of the type *cys*; *arg al* were numbered 47, 59, 7 and 62, and strains of the type *cys ylo*; *leu* were 20 and 23. The heterokaryons studied were (47 + 23), (59 + 20), (7 + 23), (62 + 20) and (59 + 23), where the *cys*; *arg al* number is written first. The two components of a heterokaryon, plus the heterokaryon itself, formed a "set" of three. Each set was studied as a single experiment (designated with a Roman numeral) and each member of the set was treated identically on the same day.

The format of the experiments is shown in Figure 1, where the "set" consists of crosses A, B and H. The component crosses of cross H are designated A_H and B_H, and the exchanges which give rise to a *cys*⁺ *pan*⁺ genotype under conditions of adenine supplementation are also shown.

RESULTS

Table 1 shows the prototroph frequencies and their manner of calculation. Column a lists the numbers of colonies prototrophic for cysteine and pantothenic acid. Column c provides an estimate of total viable spores in the population from

FIGURE 1.—The three crosses of a set, showing arrangement of markers, and exchanges selected for when adenine, leucine and arginine only are in plating medium.

* A_H and B_H here, are component crosses of the heterokaryon cross H.

** *leu* and *arg* are heterokaryon forcing markers.

*** *ylo* and *al* serve a dual function: they confirm the establishment of a heterokaryon, and they serve as nuclear (and hence cross) markers in the heterokaryon.

which the prototrophs arise; these numbers are derived from column b which lists the actual total colonies seen on the complete plates. The last column lists prototroph frequencies as percentages and these are used as an estimate of recombination in the region studied.

Within any given set, comparisons can be made between the prototroph frequencies of A-type crosses and B-type crosses, and between these frequencies and the frequency from the H-cross. If the t-test is applied to these data, significant differences are revealed in the (59 + 23) set alone. In this set there is a difference between A and B crosses at the 5% level of significance, between B and H crosses at the 1% level of significance, and between the A and H crosses at the 2% level of significance. Qualitatively, the A crosses show a lower prototroph frequency than the B crosses, and the H crosses show frequencies lower than both.

This analysis says nothing of the behaviour of the A_H and B_H crosses individually, and trends in their behaviour may be masked through considering only the overall cross-H frequencies. In order to study the behaviour of the component crosses, use can be made of the colour markers, since a yellow colony arising from cross H must have originated from the A_H component, and an albino colony must have originated from the B_H component. Table 2 shows the colour analysis of representative sample of prototrophs and colonies on complete medium. In order to compare the recombination in an A cross with recombination in an A_H cross, and a B cross with a B_H cross, the data can be manipulated in several ways, each of which can be referred to as an "index of recombination". Because

TABLE 1

Untreated prototroph frequencies observed when all strains were crossed to a common conidial parent ad-1 pan-2

Set	Protoperithecial parent	Mean No. pan-cys prototrophs from 0.1 ml stock suspension a	Mean No. colonies from 0.1 ml diluted suspension plated on complete medium b	Dilution factor	Dilution factor $\times$ b c	Prototroph frequency (percent) (a $\times$ 100)/c
(47+23)I†	47	262	1,444	15	21,680	1.21
	23	116	701	15	10,530	1.01
	HK*	31	251	15	3,760	0.82
(47+23)II	47	142	973	10	9,730	1.46
	23	50	236	10	2,360	2.12
	HK	178	1,076	10	10,760	1.65
(47+23)III	47	200	1,852	10	18,520	1.08
	23	211	1,400	10	14,000	1.51
	HK	333	2,691	10	26,910	1.24
(59+20)I	59	125	501	10	5,010	2.49
	20	146	693	10	6,930	2.14
	HK	109	189	10	1,890	5.77
(59+20)II	59	860	3,517	10	35,170	2.44
	20	285	1,951	10	19,510	1.46
	HK	290	1,816	10	18,160	1.59
(59+20)III	59‡					
	20	165.5	320.2	20	6,404	2.58
	HK	250	438	20	8,760	2.85
(59+20)IV	59‡					
	20	237	413	20	8,160	2.90
	HK	197.3	434.5	20	8,690	2.27
(7+23)I	7	88	482	10	4,820	1.83
	23	98	609	10	6,090	1.61
	HK	74	738	10	7,380	1.00
(62+20)I	62	327	1,061	10	10,610	3.08
	20	129	789	10	7,890	1.64
	HK	41	233	10	2,330	1.76
(62+20)II	62	625	3,191	10	31,910	1.96
	20	326	2,498	10	24,980	1.31
	HK	124	1,391	10	13,910	0.89
(59+23)I	59	356	597	20	11,940	2.89
	23	148.3	418.3	20	8,366	1.77
	HK	66.3	339.6	20	6,792	0.98
(59+23)II	59	372.5	521	20	10,420	3.58
	23	173	614	20	12,280	1.41
	HK	87	519	20	10,380	0.84
(59+23)III	59	587.5	862.5	20	17,250	3.40
	23	181.5	505	20	10,100	1.79
	HK	193.5	777.8	20	15,556	1.24

* Abbreviation for heterokaryon.

† Roman numerals refer to repeat numbers of sets of crosses; e.g. for set (59+20) there are four repeats.

‡ Results not obtained.

TABLE 2

Colour analysis of sample populations of prototrophic colonies and colonies growing on complete medium

Set	Experiment	Cross	Prototrophs				Colonies from complete medium			
			Size of sample analysed	Pink	Yellow	Albino	Size of sample analysed	Pink	Yellow	Albino
47+23	I	47	43	15	..	28	32	21	..	11
	II	47	32	16	..	16	32	15	..	17
	III	47	40	25	..	15	40	22	..	18
	I	23	32	18	14	..	31	15	16	..
	II	23	31	13	18	..	34	15	19	..
	III	23	35	21	16	..	33	11	22	..
	I	HK*	32	19	2	11	54	29	9	16
	II	HK	45	21	5	19	50	27	10	13
	III	HK	100	68	3	29	101	43	7	51
59+20	I	59	31	14	..	17	32	13	..	19
	II	59	32	12	..	20	32	15	..	17
	III	59†								
	IV	59†								
	I	20	30	15	15	..	32	13	19	..
	II	20	39	17	22	..	35	14	21	..
	III	20	48	33	15	..	48	31	17	..
	IV	20	50	34	16	..	49	32	17	..
	I	HK	47	32	11	4	46	21	12	13
	II	HK	90	35	21	34	89	36	18	35
	III	HK	99	44	20	35	100	57	13	30
	IV	HK	95	42	19	34	100	57	11	32
	7+23	I	7	20	14	..	6	20	9	..
I		23	20	13	7	..	20	9	11	..
I		HK	58	36	15	7	48	16	26	6
62+20	I	62	32	16	..	16	24	14	..	10
	II	62	32	19	..	13	32	16	..	16
	I	20	32	17	15	..	24	7	17	..
	II	20	32	15	17	..	29	9	20	..
	I	HK	40	18	15	7	40	22	10	8
	II	HK	79	49	15	15	78	32	33	13
59+23	I	59	44	16	..	28	47	25	..	22
	II	59	48	29	..	19	48	18	..	30
	III	59	48	27	..	21	45	22	..	23
	I	23	44	22	22	..	42	30	12	..
	II	23	47	24	23	..	45	36	9	..
	III	23	47	27	20	..	47	36	11	..
	I	HK	85	44	15	26	85	47	12	26
	II	HK	95	52	10	33	93	67	7	19
	III	HK	84	46	14	24	79	50	14	15

* Abbreviation for heterokaryon.

† Not performed.

the colour markers are on different linkage groups, most of the indices which can be formulated can not be used to compare A_H frequencies with B_H frequencies, nor A with B. (The latter may, however, be compared using the data in Table 1.) Three such indices have been calculated and are described below as indices types 1, 2 and 3.

Index Type 1: The expression "yellow prototrophs" (YP) is a measure of recombination events in an A or A_H cross, and "yellow colonies on complete medium" (YC) is a measure of the number of A or A_H meioses giving rise to these prototrophs. Thus the quotient $(YP/YC) \times 100$ is an index of recombination frequency for A-type meioses, and similarly $(AP/AC) \times 100$ is an index for B-type meioses (Table 3).

The figures used in calculating this type of index have been obtained by multiplying the numbers in columns "a" and "c" in Table 1 by the appropriate colour ratios found in the samples analysed in Table 2. Thus the B index (2.28) for set (47 + 23) experiment I is computed as follows:

$$\frac{262 \times (28/43)}{21,680 \times (11/32)} \times 100 = 2.28.$$

A full discussion of the trends shown in Table 3 and the statistical analysis of these trends, will be left until all the indices have been discussed.

Index Type 2: The calculation of this index is based on the assumption that *al-2+* and *al-2* progeny are always formed in a 1:1 ratio in the B or B_H crosses. Then if, for example, an H cross produces pink (wild type), yellow and albino individuals in a ratio of 65:10:35, it can be said that the A_H component is producing 30 pinks : 10 yellows, and the B_H component is producing 35 pinks : 35

TABLE 3

Type 1 prototroph indices(Total yellow or albino prototrophs $\times 100$) / (Total yellow or albino spores from the same cross)

Set	Experiment	A index	A_H index	Behaviour*	B index	B_H index	Behaviour*
47+23	I	0.93	0.31	D	2.28	0.95	D
	II	2.18	0.91	D	1.37	2.65	R
	III	0.88	0.54	D	0.87	0.71	D
59+20	I	1.72	5.11	R	2.30	1.72	D
	II	1.37	2.88	R	1.84	1.53	D
	III	2.28	4.43	R	(3.08)†	3.36	R
	IV	2.68	4.13	R	(3.08)†	2.54	D
7+23	I	1.02	0.47	D	1.00	0.97	D
62+20	I	1.08	2.62	R	3.70	1.54	D
	II	1.00	0.33	D	2.68	1.01	D
59+23	I	3.10	1.22	D	4.05	0.98	D
	II	3.45	1.17	D	2.26	1.44	D
	III	3.27	1.17	D	2.92	1.87	D

* D=drop in index.

R=rise in index.

† These figures are not derived from a real experiment but are means of the B index of set (59+23). The figures are omitted from the statistics.

albinos. It is evident from Table 2 that in A-type crosses, ylo^+ and ylo colonies on complete medium are not formed in a 1:1 ratio (presumably as a result of differential germination), and a simple χ^2 test confirms this. But out of 22 χ^2 tests on the segregation of $al-2^+$ and $al-2$, only one reveals a deviation from a 1:1 ratio at the 5% level of significance (set (47 + 23)I). The assumption therefore seems plausible.

The segregation of ylo^+ and ylo in an A_H cross can be calculated on the basis of the above assumption, and contingency tables may be drawn to compare the $ylo^+ : ylo$ segregation of A_H crosses with that of A crosses. These calculations give χ^2 values which lead to the following P values: for set (47 + 23) > 90%, for set (59 + 20) 50% to 70%, for set (7 + 23) 50% to 70%, for set (62 + 20) 10% to 20%, and for set (59 + 23) 70% to 80%. Thus it seems likely that in all sets, the segregation of either colour marker is not affected by the presence of the other nucleus in an H cross. As well as lending support to the type of analysis used in calculating type-1 index, this conclusion enables the calculation of the following index:

$$\frac{\text{Total prototrophs from A, B, } A_H \text{ or } B_H \text{ cross} \times 100}{\text{Total spores from the same cross}}$$

These indices are shown in Table 4.

Index Type 3: The assumption made in the calculation of Index 2 also enables the *cys-pan* region of the *cys ylo*; *leu* nucleus to be broken down into two parts, *cys-ylo*, and *ylo-cys*. Figure 1 shows that a yellow prototroph must result from an exchange in the *cys-ylo* region in an A or A_H cross. Similarly a pink prototroph from an A or A_H cross must have arisen through an exchange in the *ylo-cys* region. Thus an index may be stated as follows:

$$\frac{\text{Total pink or yellow prototrophs from an A or } A_H \text{ cross} \times 100}{\text{Total spores from the same cross}}$$

TABLE 4

Type 2 prototroph indices

 (Total prototrophs from A, B, A_H or B_H cross $\times 100$) / (Total spores from the same cross)

Set	Experiment	A index	A_H index	Behaviour of type A index	B index	B_H index	Behaviour of type B index
47+23	I	1.01	0.63	D	1.21	0.96	D
	II	2.12	0.54	D	1.46	2.69	R
59+20	I	2.14	11.00	R	2.49	1.74	D
	II	1.46	1.83	R	2.44	1.53	D
	III	2.58	2.09	D	...	...	...
	IV	2.90	1.79	D	...	...	...
7+23	I	1.61	1.01	D	1.83	0.97	D
62+20	I	1.63	1.91	R	3.08	1.54	D
	II	1.30	0.83	D	1.96	1.02	D
59+23	I	1.77	0.98	D	2.98	0.98	D
	II	1.41	0.43	D	3.58	1.43	D
	III	1.79	0.86	D	3.40	1.87	D

Of course, the yellow index obtained from this expression will measure the same recombination as the A or A_H type 1 index.

Trends in behaviour of indices. The qualitative behaviour of each index in each experiment is shown in the tables as a D (drop) or R (rise) on entering a heterokaryon.

Table 3 reveals three trends: (i) In all the heterokaryons in which it is present, the strain *cys ylo; leu* #23 shows a lower A_H index than an A index. Taken together these figures result in a t value which leads to $P < 1\%$ (6 df). (ii) Conversely the A_H indices of strain *cys ylo; leu* #20 are consistently higher than their respective A indices, and on comparison in a t-test reveal a P value $< 5\%$ (5 df). (iii) As a general rule the B_H indices of all the *cys; arg al* strains are lower than their respective B indices, the t-test giving $P < 5\%$ (10 df).

Table 4 reveals similar trends both for strain #23 ($P < 1\%$, 5 df only as (47 + 23) III cannot be calculated), and the *cys; arg, al* strains in general ($P < 2\%$, 9 df). But for this type of index, the indices for strain #20 show no significant trend.

Table 5 shows for all sets a lower A_H (pink) index than the A (pink) index, the t-test comparison resulting in $P < 1\%$ (10 df as (59 + 20) experiment I was omitted from the statistical analysis). The A_H (yellow) indices are (in the same manner as the A_H indices of Table 3), mostly smaller than the A (yellow) indices where strain #23 is the *cys ylo; leu* component ($P < 1\%$, 5 df), but the *cys ylo; leu* #20 indices show no significant difference in the statistical analysis although qualitatively the behaviour is the same as in Table 3.

In all the tables, the trends indicated above are shown most markedly in the (59 + 23) sets. The trends are summarized in Figure 2.

TABLE 5

Type 3 prototroph indices

(Total pink or yellow prototrophs from A or A_H cross $\times 100$)/(Total spores from same cross)

Set	Experiment	A (pink) index	A_H (pink) index	Behaviour of type A (pink) index	A (yellow) index	A_H (yellow) index	Behaviour of type A (yellow) index
47+23	I	0.62	0.51	D	0.48	0.13	D
	II	0.89	0.15	D	1.23	0.38	D
59+20	I	1.05	7.90	R	1.05	3.10	R
	II	0.64	0.08	D	0.82	1.74	R
	III	1.78	0.65	D	0.81	1.44	R
	IV	1.98	0.53	D	0.93	1.26	R
7+23	I	1.05	0.67	D	0.56	0.35	D
62+20	I	0.87	0.81	D	0.77	1.09	R
	II	0.63	0.58	D	0.69	0.25	D
59+23	I	0.89	0.53	D	0.89	0.44	D
	II	0.72	0.28	D	0.69	0.15	D
	III	1.03	0.53	D	0.77	0.34	D

FIGURE 2.—Summary of prototroph index behaviour of specific regions of linkage group VI when under the influence of a dissimilar nucleus.

DISCUSSION

The trends analysed above are consistent in their occurrence, but before accepting them as manifestations of real recombination behaviour, several points must be considered.

No selective technique is available for the detection of colonies pseudowild for *pan* and *cys*, and the prototroph data have not been corrected for their presence. However, several factors indicate that pseudowild production is not appreciable in the strains used. First, in Table 2, a pink to yellow prototroph ratio considerably in excess of unity would be expected if nondisjunction were prevalent for linkage group VI. This is found to any significant extent in only two of the 13 *ylo* × + crosses analysed (set (59 + 20) III and IV). Secondly, although there is no way of estimating PWT production for linkage group VI in the *cys*; *arg al* strains, the data in Table 2 show no indication of nondisjunction for linkage group I on the basis of the criterion used above.

100% germination of ascospores is rarely obtained in *Neurospora*, but different strains vary in a consistent manner in their germination behaviour, suggesting gene control. A contingency table has been used to compare germination figures for all the (59 + 23) sets and the sets (59 + 20) I and II (for which data are available). The analysis shows no significant difference between the values, which are consistently around 82%. It seems unlikely, in these sets at least, that the trends are due to differences in gene control of germination.

Table 1 shows a variation in the prototroph frequency in repeats of some of the crosses. This problem is often met in prototroph analysis and the cause is not fully understood. However, all three experiments of any given set have been performed on the same day using identical procedures and medium in order to eliminate this problem as much as possible. Also, the use of the t-test, which compares only members of a set performed on the same day, helps to reduce the importance of such a phenomenon.

For practical reasons, the samples isolated in Table 3 were not large compared with total number of spores used, and extrapolation of ratios detected from the

sample to the larger figures is questionable. However, as stated above, for any given cross the behaviour of the indices revealed using such a procedure is remarkably consistent when the experiments are repeated.

Complementation between the *arg* and *leu* genes in the heterokaryons has made it necessary to use differently supplemented medium in the H cross tubes to that in the A and B cross tubes. However, the level of supplementation used was so low as to result in no significant difference in pH between the two media, and it is unlikely that the mere presence of arginine and leucine in the A and B crosses could account for recombination differences between these and the H crosses.

With the exception of the rise in prototroph frequency over the *cys-ylo* region of *cys ylo; leu* #20, the general conclusion which can be drawn from the data is that the presence of another type of nucleus in a heterokaryon has the effect of decreasing the efficiency of the recombination process. This mutual inhibition of recombination suggests that differences in recombination cannot be attributed to structural difference in these strains and supports the concept of the control of recombination by gene products. The interesting finding is the fact that the action of this gene product (or gene products) is of an inhibitory nature. The *rec*⁺ allele identified by JESSOP and CATCHESIDE too appeared to be inhibitory in nature, its presence in either parent causing a low frequency of *his-1 prototrophs*. The suggestion of JESSOP and CATCHESIDE that *rec*⁺ produces an enzyme which patrols the DNA, repairing breaks that may be associated with recombination, is worth consideration in the light of the above data. Internuclear exchange of the products of *rec*⁺-type genes might explain the joint lowering of the prototroph indices.

If in fact inhibitory enzymes are concerned, it is difficult to find a place for them in a model such as that of WHITEHOUSE and HASTINGS (1965), except at the level of DNA-repair. There is evidence from work on bacteria that enzymes exist which are responsible both for the repair of damage caused by ultraviolet irradiation and for recombination processes (CLARK and MARGULIES 1965; STRAUSS and REITER 1965). However, in terms of the WHITEHOUSE and HASTINGS model, these would most likely act at the level of hybrid DNA correction, as the ultraviolet-sensitive strains are recombination deficient.

All the data reported so far in this paper are from crosses using *ad-1 pan* as the conidial (fertilizing) parent. One (59 + 23) set has been investigated using *ad-1 pan-2* as the protoperithecial parent. Although the data on this set are not yet extensive enough to present statistically, the results show sufficient resemblance to the reciprocal crosses to suggest two points: (i) It is unlikely that the behaviour of the indices is due to cytoplasmic effects that are not directly related to nuclei. (ii) The internuclear exchange of gene products takes place, and has its effect, before meiosis, since the male fusion nucleus (provided by the heterokaryon) is no longer in cytoplasmic contact with its partner nucleus at the time of meiosis. Furthermore, in the experiments using *ad pan* as the conidial parent, it seems unlikely that the female fusion nucleus is in cytoplasmic contact at the time of meiosis with its heterokaryotic partner nucleus, because of the insular nature of the protoperithecium. In this connection, however, mention must be made of

several cases in which genetically mixed perithecia have been reported in crosses involving heterokaryons (SANSOME 1947; NAKAMURA and EGASHIRA 1961; WEIJER and DOWDING 1960).

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SUMMARY

Two groups of strains have been isolated, *cys-2 ylo*; *leu-3* and *cys-2*; *arg-6 al-2*. The frequencies were determined at which these strains produce spores prototrophic for cysteine and pantothenic acid when crossed to a common conidial parent *ad-1 pan-2*.—When two strains, one from each group, are united to form a heterokaryon, the prototroph frequency of each individual component cross decreases when the heterokaryon is crossed to *ad-1 pan-2*. One exceptional strain has been found which shows an increase in prototroph frequency in one region of the interval studied, but a decrease in an adjacent region.—The results can be interpreted as evidence for a cross-exchange of gene products between the nuclei of the heterokaryons.

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