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Apparent Self-Fertility in *Neurospora crassa*

By P. G. MARTIN

Genetics Department, University of Adelaide, South Australia

SUMMARY: Single ascospores which gave rise to temporarily self-fertile cultures were found in *Neurospora crassa*. Genetical and cytological evidence suggested that these ascospores were disomic for the chromosome containing the mating-type locus, the disomy having arisen following partial desynapsis at meiosis and unequal distribution of univalents. At some time after germination, the nuclei reverted to the haploid state. If the disomic ascospore had been heterozygous at the mating-type locus, two sorts of haploids might occur and fertility probably resulted from mating between these.

The genes *pdx* (pyridoxineless) and *pdx-p* (pyridoxineless, ammonia-sensitive) in *Neurospora* are either allelic or pseudo-allelic (Mitchell, 1955). Pseudo-wild type strains (Mitchell, Pittenger & Mitchell, 1952; Pittenger, 1953, 1954) occur among the pyridoxine-independent segregants from the cross *pdx co A* × *pdx-p a*; *co* is a colonial marker gene approximately five units distal to *pdx* and *pdx-p*. Although these grow in the absence of pyridoxine, they segregate approximately 50 % pyridoxineless spores in crosses to wild type. This is thought to result from the spores being disomic for the chromosome carrying the pyridoxineless loci, the disomic nuclei being pyridoxine-independent but not functioning in fertilization; only haploid derivatives of the disomic are fertile (Mitchell *et al.* 1952; Pittenger, 1953, 1954).

Prototrophic segregants from this cross were classified by outcrossing to wild type strains (extracts from Emerson *A* and *a*) and scoring the progeny. Apparent self-fertile strains were observed in the progeny of four out of sixteen of these crosses; in the sequel the abbreviation A.S.F. will be used.

This paper describes the origins of the A.S.F. strains which were investigated and the results of genetical and cytological investigation of them.

METHODS

Strains. The strains of *Neurospora crassa* used were *pdx*: (37803), *pdx-p* (44602); *co* (70007), (Barratt *et al.* 1954). These were descendants of stocks obtained by Professor D. G. Catcheside from Professor G. W. Beadle. The wild type strains used were Emerson *A* and *a* which were extracts from Emerson *A* (5296) × Emerson *a* (5297) and Beadle *a*, an extract from Beadle 25*a* × Beadle 1*A*. The genes *pdx*, *pdx-p* and *co* are not in the same linkage group as the mating-type locus.

Media. The methods used for cleaning glassware, making and sterilizing media were, generally, those recommended by Ryan, Beadle & Tatum (1943). The minimal medium used was that of Westergaard & Mitchell (1947) while, to distinguish *pdx-p* from *pdx*, the 'm/15 phosphate medium' of Strauss

(1951) was used: strain *pdx-p* grows well on the latter while strain *pdx* does not grow at all. Supplements of vitamins and other growth substances were used at the concentrations recommended by Beadle & Tatum (1945).

Methods. Methods of inoculation, making crosses, ascospore isolation from ordered asci and germination and incubation were generally as recommended by Beadle & Tatum (1945). Once the possibility of self-fertility was suspected and exclusion of the possibility of contamination became even more important, care was taken that tubes in which ascospores had been incubated were not opened until after perithecium formation might have been expected. Twenty days were allowed and strains classified as A.S.F. developed perithecia before this time. Where it was important to obtain unequivocal results from mating-type tests, each ascospore isolation was tested against standard strains of both mating types. The strains used were Emerson *A* and Beadle *a*, the latter being preferred to Emerson *a*, which is slow in forming protoperithecia. For positive identification of mating-type, perithecia which contain ascospores in one cross, but not in the other, were required.

Cytology. The techniques used were similar to those described by Singleton (1953) except that acetic acid ethanol was replaced by a fixative described by Newcomer (1953). This appeared to clear the cytoplasm better and gave good fixation of chromosomes. Fixed material was hydrated in stages and then hydrolysed in *N*-hydrochloric acid at 60° for 6 min. The stain used was 2% (w/v) synthetic orcein (G. T. Gurr, London) in 60% (v/v) acetic acid in water. About 6 perithecia were squashed in a drop of stain on an acid-washed slide. A Zeiss microscope fitted with an apochromatic objective ($\times 90$, N.A. 1.30) and an aplanatic condenser (N.A. 1.40) was used. Photographs were taken with a 35 mm. Leica camera and Ilford FP3 film.

RESULTS

Origin of A.S.F. strains. In order to classify prototrophs from the cross *pdx co A* $\times$ *pdx-p a*, it was necessary to classify their progeny from crosses to wild type strains. Apparent self-fertile strains were observed among the wild type segregants from four of these crosses. The nature of the parents and designation of the A.S.F. strains are shown in Table 1. At first these were attributed to contamination by causes unknown. It was observed, however, that the perithecia contained very few ripe ascospores; but a single ripe ascus was observed and dissected from A.S.F. 1. The spores were germinated in tubes of minimal medium which, after heat treatment, were not opened, thus ensuring freedom from contamination. Spore number 4 did not germinate and of the seven remaining spores six were of mating type A. Spore number 1 produced an A.S.F. strain, henceforth referred to as A.S.F. 9. All seven segregants were pyridoxine-independent, but A.S.F. 9 was subsequently shown to produce *pdx-p* progeny.

This most abnormal result suggested that A.S.F. strain 1 was indeed self-fertile. Accordingly, A.S.F. strains 1-8 were re-examined. Although many perithecia were formed, they contained mostly aborted ascospores. This extreme infertility

Table 1. *Origin and designation of A.S.F. strains*

Prototrophic parent	Other parent	Frequency of A.S.F. strains in progeny	Designated A.S.F. number
Pseudo-wild type	Emerson <i>A</i>	4 in 24	1-4
Pyridoxine-independent, non-colonial	Emerson <i>A</i>	1 in 19	5
Pyridoxine-independent, colonial	Emerson <i>A</i>	1 in 20	6
Pyridoxine-independent, colonial	Emerson <i>a</i>	2 in 41	7-8

Table 2. *Segregations observed in asci from A.S.F. strains*

		Segregation of <i>pdx-p</i> , <i>co</i> and mating type					
		<i>pdx-p</i>		<i>co</i>		Mating type	
A.S.F. No.	Progeny	First division	Second division	First division	Second division	First division	Second division
1	1 <i>pdx-p</i> :1 +	2	0	.	.	1	1
2	1 <i>pdx-p</i> :1 +	2	1	.	.	2	1
3	All +	.	.	.	.	0	2
5	1 <i>co</i> :1 +	.	.	1	2	0	3
6	1 <i>co</i> :1 +	.	.	2	1	2	1
7	All +	.	.	.	.	3	0
8	All +	.	.	.	.	2	2
		4	1	3	3	10	10

suggested that A.S.F. 1-8 all had similar origins and that this was not contamination. A few complete ripe asci were found and dissected with the results shown in Table 2. Since no A.S.F. strains were found among the asci, eighty-three random ascospores from the selfing of A.S.F. 9 were examined and were classified as follows:

	A	a	No reaction	Total
<i>pdx-p</i> +	11	15	12	38
<i>pdx-p</i>	15	21	7	43
	26	36	19	81

The segregation ratios of *pdx-p*:*pdx-p*⁺ and of mating type, where it could be scored, were both approximately 1:1. In addition there were two A.S.F. strains, both pyridoxine-independent, and these have been designated 10 and 11.

All the progeny of A.S.F. 10 and 11 were *pdx-p*⁺. Four ordered asci out of thirteen from A.S.F. 11 contained new A.S.F. strains designated 12-15 (Table 4).

The properties of A.S.F. strains

(1) *General properties.* Perithecia resulting from selfing first appeared later than expected in an ordinary cross, i.e. when 15-20 days old. In subsequent sub-cultures, however, they appeared after the expected 5 or 6 days. Few ripe ascospores were formed—usually only about 30/perithecium and a completely ripe ascus occurred only in about one in four perithecia. Most ascospores aborted and all combinations of aborted and ripe ascospores were seen. No A.S.F. strain retained its self-fertility for more than two or three subcultures.

(2) *Genetical properties.* In A.S.F. 10, mating type segregated at the second division in ten out of fourteen asci and in A.S.F. 11 in five out of thirteen asci. These figures, combined with the data for A.S.F. 1-8 in Table 2, give a total of twenty-five second-division segregations of mating type in forty-seven asci from selfed A.S.F. strains. The 95 % fiducial limits for this observed proportion are 17.9/47 and 32.1/47 so that the observations differ significantly from the normal expectation of 14 %, i.e. 6.6/47.

Table 3. *Ascus types in progeny of A.S.F. strains*

Parent	Ascus type	Segregation of mating type	Segregation of growth rate	No.
A.S.F. 10	Ditype	First division	First division	9
	Ditype	Second division	Second division	3
	Tetratype	Second division	First division	1
A.S.F. 11*	Ditype	First division	First division	4
	Ditype	Second division	Second division	3
	Tetratype	Second division	First division	3
	Tetratype	Second division	Second division	1

* These eleven asci include nos. 1, 2 and 4 in Table 4.

Asci from A.S.F. 10 and 11 each showed a distinct single-factor segregation for growth rate. However, this may have had different causes in the two cases, for in A.S.F. 10, but not A.S.F. 11, the slow growth rate was accompanied by a fluorescent medium (under u.v. radiation) and the formation of melanin. The factor for growth rate was in both cases linked to mating type and in both cases *A* was usually the faster. Ascus types are shown in Table 3. In both cases it would appear that the gene affecting growth rate had its locus between the centromere and the mating-type locus.

Table 4. *Composition of some abnormal asci from selfing in A.S.F. 11*

Ascus no.		Spore no.							
		1	2	3	4	5	6	7	8
1	Mating type	<i>a</i>	A.S.F. 12	<i>A</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>a</i>	<i>a</i>
	Growth rate	Slow	Slow	Slow	Slow	Fast	Fast	Fast	Fast
2	Mating type	<i>a</i>	<i>a</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>A</i>	A.S.F. 13	<i>a</i>
	Growth rate	Slow	Slow	Fast	Fast	Slow	Slow	Fast	Fast
3	Mating type	Aborted	Aborted	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	A.S.F. 14	Aborted
	Growth rate	.	.	Slow	Slow	Slow	Slow	Fast	.
4	Mating type	<i>a</i>	<i>a</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>A</i>	Aborted	A.S.F. 15
	Growth rate	Fast	Fast	Fast	Fast	Slow	Slow	.	Slow
5	Mating type	<i>a</i>	<i>a</i>	<i>a</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>a</i>	Aborted
	Growth rate	Slow	Slow	Slow	Fast	Fast	Fast	Slow	.

Asci containing, for example, seven mature ascospores and one hyaline aborted spore, suggested that members of a spore pair could differ. This was confirmed in the four asci containing A.S.F. 12-15, and in another ascus, the composition of which, with respect to mating type and the growth-rate factor, are shown in Table 4.

An examination of these asci suggests that A.S.F. strains nos. 12, 13 and 15 should have been *a* while A.S.F. 14 should have been *A*. When these strains reverted to normal they did not necessarily revert to the mating-type expected from position in the ascus. Thus while A.S.F. 13, 14 and 15 reverted to their 'correct' mating types, A.S.F. 12 reverted to *A* instead of *a*. A.S.F. strains 9, 10, and 11 whose 'correct' mating types were uncertain, reverted to *A*, *A* and *a*, respectively. Thus A.S.F. strains revert to a single mating-type, but this is not necessarily that which, from position in the ascus, would be expected; moreover, they seem to revert equally frequently to *A* and *a*.

(3) *Cytological properties.* A complete cytological analysis of self-fertility was not carried out and most observations were confined to the more easily observable stages in the ascus, viz. diakinesis, metaphase I and anaphase I. With orcein staining the spindle and nucleolus were not visible. This, together with the fact that in minute chromosomes it is difficult to distinguish singleness from doubleness, sometimes made unambiguous interpretation of a configuration difficult.

The original cross which gave rise to one parent of the first A.S.F. strains was *pdx A* $\times$ *pdx-p a*. From this cross twenty-two asci were observed at diakinesis or metaphase I. In most of these meiosis appeared to be quite normal (e.g. Pl. 1, fig. 1) but in one, eight bodies were present (Pl. 1, fig. 2). These were presumably six bivalents and two univalents. Of ten anaphase I and early telophase I stages, most appeared to be quite normal (e.g. Pl. 1, fig. 3) but in one there appeared to be six bodies at one pole, five at the other, with three laggards (Pl. 1, fig. 4). Presumably five bivalents disjoined normally, leaving three univalents on the equatorial plate while the other univalent was carried to one pole.

If the interpretations of Pl. 1, figs. 2 and 4 are correct, it seems probable that univalents are occasionally formed in this cross. The distribution of these might give rise to aneuploid ascospores which, if disomic for the *pyridoxineless* chromosome, would give rise to the pseudo-wild type strains observed.

A.S.F. strains 1-4 were found in the progeny of the cross of one of these pseudo-wild type strains to Emerson *A*. Among five diakinesis metaphase I configurations and four anaphase I configurations, no abnormalities were detected. However, if univalents were also formed in crosses of this kind and others, an aneuploid ascospore disomic for the mating-type chromosome might give rise to A.S.F. strains.

Plate 1, fig. 5 shows meiosis in an ascus resulting from selfing in A.S.F. strain 11. The stage appears to be early anaphase I, but there are more than the expected seven bodies going to each pole. There appear to be twelve bodies at one pole, nine at the other and one between the two groups. This figure suggests either that the original zygote was polysomic or that the zygote was diploid but asynaptic and univalents divided at metaphase. Thus if one bivalent was formed and disjoined normally, four univalents did not divide and eight did, the result would be twenty-two bodies at anaphase and these could be distributed as in Pl. 1, fig. 5.

Other asci from the same selfing appeared to be abnormal. Pl. 2, fig. 6, which

contains fifteen bodies, appears to be a stage earlier than Fig. 5. The fifteen bodies might arise from fourteen univalents, one of which has already divided. Plate 2, fig. 7, contains twelve bodies, possibly two bivalents and ten univalents. In Pl. 2, fig. 8, prophase II appears to have seven bodies at one end and fourteen at the other. This might be normal anaphase II, progressing at the latter end, but it does not appear to be so. In Pl. 2, fig. 9, an ascus from selfing in A.S.F. 10, there appear to be eight bodies at late diakinesis, presumably six bivalents and two univalents.

Altogether fourteen diakinesis—metaphase I configurations and six anaphase I configurations were observed in selfings of A.S.F. strains 10, 11 and 12. Of these only five and two, respectively, appeared to be normal. Although the remainder (see figs. 5–9) were sometimes open to alternative explanations, no similar cases were observed in the cross *pd $\bar{x}$ -p a* $\times$ *Emerson A* and only on two occasions in the cross *pd $\bar{x}$ A* $\times$ *pd $\bar{x}$ -p a* (see Pl. 1, figs. 2 and 4). It is therefore concluded that meiosis in selfings was very often irregular and that the interpretations of the configurations are similar to those given.

Similar irregularities were observed in a cross between A.S.F. strains 10 and 11. Plate 2, fig. 10, shows an ascospore containing eleven bodies. This might be early anaphase with only four chromosomes disjoined, but it does not appear to be so.

Segregants from selfings of A.S.F. strains were crossed to wild-type strains for mating-type tests and some of these crosses were examined cytologically. Most configurations appeared to be normal but a few showed evidence of univalent formation. Plate 2, fig. 11, is an excellent example of this, there being five unmistakable laggards at telophase I.

DISCUSSION

It seems probable that the behaviour of A.S.F. strains can be explained on the hypothesis that they are disomic for the mating-type chromosome, and probably others, and that the disomic condition is not stable but reverts to haploidy.

Viable ascospores, disomic for the mating type chromosome and heterozygous for mating type might be formed in several ways.

(1) Distribution of univalents during meiosis in a zygote which either has more than two mating-type chromosomes or else has two but is asynaptic or desynaptic. Asynapsis implies that the homologous chromosomes do not pair at zygotene, whereas desynapsis implies that they do, but later fall apart. In desynapsis there is opportunity for chiasma formation and therefore crossing over. Genetical evidence for this has been obtained in asynaptic tomato (Soost, 1951) and in maize (Rhoades, 1947).

(2) Non-disjunction at anaphase of a division reductional for mating type (either anaphase I or II) in a diploid. One would expect this to cause abortion of other spores in the ascus.

(3) Non-disjunction in an interchange heterozygote.

Since the second and third hypotheses would require abortion of some spores in an ascus containing an A.S.F. strain, they are rendered unlikely by such asci as numbers 2 and 4 in Table 4. Recombination between mating-type and growth-rate in each of asci numbers 1, 2 and 4 also indicates that, if the A.S.F. strain is caused by distribution of univalents, then the univalents are formed after crossing-over has taken place. Either the zygote is desynaptic or a univalent has occurred with an homologous bivalent, or a false univalent has been derived from a trivalent.

Assuming that an ascospore giving rise to a self-fertile strains is disomic for the mating-type chromosomes and heterozygous for mating type, i.e. Aa , the self-fertile reaction presumably follows the formation of haploid nuclei by rare chromosome loss and selection for this haploid. The mating would then be either $Aa \times A$, $Aa \times a$ or $A \times a$. If the mating is between haploid cells, i.e. $A \times a$, unless the zygote is desynaptic, there is no reason to expect either A.S.F. strains in the progeny or abnormal segregations such as in the ascus containing A.S.F. 9 or those shown in Table 4. Therefore, it seems probable that the mating-type reaction is between a disomic and monosomic or else that it is between two monosomics; and the resulting zygote is desynaptic.

There are three objections to the hypothesis that the mating-type reaction is between a disomic and monosomic. If the mating-type reaction is $Aa \times A$ (or $Aa \times a$) there is no obvious way to account for a high frequency of second division segregation for mating type. Secondly, A.S.F. strains should be very frequent in the progeny. Thirdly, if the hypothesis that pseudo-wild type strains originate as disomics is correct then it is also correct that disomics for five of the linkage groups, including the one containing the mating type (Pittenger, 1954), do not function in fertilization; this hypothesis requires that they should. This leaves the hypothesis that the mating reaction in an A.S.F. strain is $A \times a$ and the resulting zygote is desynaptic. The cytological observations would agree with this hypothesis because it allows a maximum of fourteen bodies at each pole at anaphase I. This has been observed by Catcheside (1939) in an asynaptic *Oenothera* which also has a diploid complement of fourteen chromosomes. A high proportion of second-division segregation for mating type means that most of these asci must arise in the following manner. Both univalents divide at the first division of meiosis and are included in telophase nuclei. They do not divide at the second division of meiosis but are distributed so that each telophase II nucleus has one mating-type chromosome. If daughter univalents should divide at the second division of meiosis then they would have replicated once more than, and out of step with, other strands and it is not unreasonable to suppose that they might not replicate at the third division in the ascus. This would result in dissimilar members of a spore pair. For example, the ascus which contained A.S.F. 9 might have arisen as follows. Both univalents divided at anaphase I. One of the a daughter univalents was lost; the other was not but did not divide at anaphase II. Both A daughter univalents were included in telophase I nuclei and divided again at anaphase II and anaphase III. Thus all spores contained A and spore 1

also contained *a*. Spore number 2 probably also contained *a* but self-fertility was not detected.

This hypothesis would adequately account for all observed properties of A.S.F. strains. Simultaneously disomy for more than one chromosome would be expected in some ascospores; this has apparently been observed for the *pdx-p* and mating-type chromosomes in A.S.F. 1, 2, 5 and 6.

Vegetative recombination between the mating-type locus and the unlinked *pdx-p* locus in such an A.S.F. strain should be easily observable in conidia or hyphal tips. A demonstration of this would provide good evidence to support the hypothesis, but it has not been attempted. It is suggested that a similar explanation will account for the formation of pseudo-wild type strains, i.e. that the cross *pdx* × *pdx-p* is partially desynaptic giving rise to occasional disomics. Some cytological evidence for univalent formation in this cross has been presented. That segregants from this cross formed one parent of the crosses from which A.S.F. strains were derived is probably significant but not the whole story. The other parent was either Emerson *A* or Emerson *a*. The author has observed A.S.F. strains in the progeny of the following crosses:

33757 *a* (*leucineless*) × Emerson *A*,

44206 *A* (*adenineless*) × Beadle *a*.

Mr P. Fitzgerald of this department has observed them in the cross Emerson *A* × Emerson *a*, his stocks having been obtained from the author. Moreover, since pseudo-wild type formation is so widespread (Pittenger, 1954), it seems probable that, if desynapsis is the cause, it is common in laboratory stocks of *Neurospora*.

There are only two reports in the literature of self-fertility in *Neurospora crassa*. Lindegren (1934) attributed it to heterocaryotic ascospore formation. Pateman (1955) observed it but did not make a further report. Probably self-fertility has been observed frequently but attributed to contamination. It is unlikely to be of great use genetically because A.S.F. strains retain their self-fertility for such a short period and are very sterile. However, it should be possible to derive strains of the opposite mating-type which are isogenic (at least temporarily) in all but the mating type chromosome.

Asynapsis has been reported in a wide variety of plants but there is usually no evidence that it is not desynapsis. Asynapsis is frequently inherited as a single gene effect. There is no apparent reason why inherited asynapsis or desynapsis should not occur in *Neurospora*. Possibly the extreme sterility observed in A.S.F. strains is caused by homozygosity for a gene or genes controlling the desynapsis while in other crosses, such as those giving rise to pseudo-wild type strains, the effect is only partial.

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EXPLANATION OF PLATES

PLATE 1

- Figs. 1, 1a. Normal metaphase I in an ascus from the cross *pdx A* × *pdx-p a*. Magnification, × 1200.
- Figs. 2, 2a. Metaphase I in cross *pdx A* × *pdx-p a* with eight bodies present. Magnification, × 1200.
- Figs. 3, 3a. Normal anaphase I in cross *pdx A* × *pdx-p a*. Magnification, × 1200.
- Figs. 4, 4a. Anaphase I in cross *pdx A* × *pdx-p a* showing laggards. Magnification, × 1200.
- Figs. 5, 5a, 5b. Early anaphase I in selfing of A.S.F. 11. Twenty-two bodies present. Magnification, × 1200. Fig. 5a was taken in a lower focal plane than Fig. 5.

PLATE 2

- Figs. 6, 6a. Metaphase I (approximately) in selfing of A.S.F. 11. Fifteen bodies present. Magnification, × 1200.
- Figs. 7, 7a. Metaphase I (approximately) in selfing of A.S.F. 11. Twelve bodies present. Magnification, × 1200.

Figs. 8, 8*a*. Prophase II (approximately) in selfing of A.S.F. 11. Fourteen bodies in one group, seven in the other. Magnification, $\times 900$.

Figs. 9, 9*a*. Metaphase I in selfing of A.S.F. 10. Eight bodies present. Magnification, $\times 1200$.

Figs. 10, 10*a*. Ascospore from cross of ASF $10 \times$ A.S.F. 11. Probably metaphase with eleven bodies present. Magnification, $\times 1200$.

Fig. 11, 11*a*. Telophase I in cross of a segregant from an A.S.F. strain to a wild type strain. Magnification, $\times 1200$.

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