ASEXUAL SELECTION IN NEUROSPORA CRASSA[1]

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HETEROCARYOSIS, the association of genetically different nuclei in a single cytoplasmic unit, has been recognized in asexually reproducing fungi as a mechanism of natural variation since the work of HANSEN and SMITH (1932) on the "dual phenomenon." They discovered that wild isolates of *Botrytis cinerea* were unstable upon single conidial transfer, the wild isolate giving rise to two stable "conidial" and "mycelial" strains, as well as the unstable, intermediate wild type. Later investigations (HANSEN 1938) showed that this behavior was widespread among the Fungi Imperfecti. In 1942, HANSEN and SNYDER discovered the dual phenomenon in the sexually reproducing Ascomycete, *Hypomyces solani*. By standard genetic tests, it was shown that the "conidial" and "mycelial" characters were controlled by a single pair of alleles, and that the dual phenomenon was doubtless the result of the assortment of two different nuclear types of a heterocaryon during the formation of multinucleate conidia.

The investigations of DODGE (1942), and of BEADLE and COONRADT (1944) with various mutant strains of Neurospora species have revealed a great deal concerning the physiological consequences of heterocaryosis. In most cases, it was found that heterocaryotic mycelia, constituted of two nuclear types carrying non-allelic mutations would display a normal phenotype. They felt that the mutant gene carried by one nuclear type was compensated for by the action of its wild type allele in the other nuclear type. Though not strictly comparable with dominance in diploid systems, the complementary action of wild type genes over their mutant alleles in heterocaryons indicated a dominant-recessive relationship.

The occurrence of the dual phenomenon in nature and the experimental investigations of heterocaryosis indicate that filamentous fungi possess a more adaptable genetic system than do unicellular haploid organisms. Because many species of fungi reproduce by multinucleate conidia, a heterocaryotic condition may be maintained not only during the growth of mycelia, but also in their asexual reproduction. The plasticity of such a system would be somewhat akin to that of diploids, because a reserve of variability could accumulate in a nuclear population by mutation, shielded from adverse selection by more advantageous genomes.

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The changes in the nuclear proportions of heterocaryons during conidial transfers, therefore, may be investigated, and such a study may be used to construct a model of natural selection in fungi which reproduce asexually by means of multinucleate conidia. Asexual reproduction in *N. crassa* involves uni- and multinucleate conidia, among which nuclei of different types may be distributed almost randomly (PROUT, HUEBSCHMAN, LEVENE and RYAN 1953; ATWOOD and MUKAI 1955; and KLEIN 1958). A heterocaryon may therefore produce conidia of three classes; two of which contain only one type of nucleus (homocaryotic) and one of which contains both (heterocaryotic). With a heterocaryotic system constituted of two nutritionally deficient nuclear types, it is possible to select against a homocaryotic conidial type by omitting its nutritional requirement. By studying the changes of nuclear proportions over many serial conidial transfers, the factors governing these changes may be determined. The experiments reported here were designed to elucidate these factors and to indicate the extent to which nuclear selection in asexually reproducing heterocaryons of *N. crassa* is comparable with the pattern of genic selection in populations of sexually reproducing diploid organisms.

MATERIALS AND METHODS

The homocaryotic strains of *N. crassa* from which the heterocaryons were synthesized were derived from single ascospore isolates of the genotypes *pan, al-1 A* (5531, 4637; pantothenic acid, albino) and *nic-2, al-2 A* (4540, 15300; nicotinic acid, albino). The mutations are described in the index of BARRATT, NEWMEYER, PERKINS and GARNJOBST (1954).

The medium used for the experimental transfers of the heterocaryons was the minimal medium of RYAN (1950). This will be referred to as "standard medium." The medium used in plating conidia for estimations of nuclear ratios was a modified form of that of WESTERGAARD and MITCHELL (1947), and contained one percent sorbose and 0.1 percent sucrose. Either medium, when unsupplemented, will be described as "minimal." Where nutritional supplements were appropriate, they were added in the following concentrations: calcium pantothenate, 2 μg per ml medium; nicotinic acid, 2 μg per ml; nicotinamide, 2 μg per ml; and l-lysine, 50 μg per ml.

The heterocaryons of *pan, al-1* and *nic-2, al-2* used in the experiments were formed by the method of PITTENGER, KIMBALL, and ATWOOD (1955). Heterocaryons constituted of these nuclear types grow without either growth supplement at a rate indistinguishable from that of wild type. Mycelia of this type form three types of conidia: *pan, al-1* homocaryons, *nic-2, al-2* homocaryons, and heterocaryons, containing both nuclear types. The present experiments were designed to select against one or the other of the homocaryotic conidial types during conidial transfers. This was accomplished by establishing eight heterocaryons and subsequently transferring four of them serially on pantothenate supplemented standard medium (PAN lines), and four of them on nicotinic acid supplemented medium (NIC lines). Selection was maintained over many serial transfers on

agar slants in 18 × 150 mm culture tubes, and the changes in nuclear ratio were recorded. The term *transfer* will be used for the most part; for clarity in some cases, the term *generation* will refer to the heterocaryon arising from a given transfer. The regular transfers of the lines were performed by taking a suspension of conidia from a heterocaryon, filtering it through glass wool, and diluting it to a concentration of 1000 to 6000 conidia per ml. 0.1 ml of the dilution was pipetted evenly over the slants. After four days, the resulting mycelium had developed a rich conidial mass, and the procedure was repeated.

At each transfer, determinations of the frequencies of the three conidial types from each heterocaryon were made in order to estimate the nuclear proportions. This was done by plating aliquots of the conidial suspension used for transfers in triplicate on three types of media: minimal plating medium + lysine, minimal + pantothenate + lysine, and minimal + nicotinamide + lysine. (Lysine was previously found to improve the germination of conidia in this medium.) By appropriately subtracting colony counts on minimal medium from those on supplemented media, the frequencies of the three conidial types were obtained. At disparate nuclear ratios, where it was expected that heterocaryotic conidia and homocaryotic conidia of one type would be rare, the number of conidia plated in minimal and in medium supplemented to select the rare homocaryon was increased tenfold. This allowed a more accurate determination of the proportions of minority conidial types.

The average number of nuclei per conidium, which in most cases lies between two and three, was determined at each transfer by counting 500 stained conidia (HUEBSCHMAN 1952). The conidia of one of the PAN lines and of one of the NIC lines were sampled at each transfer, and the average nuclear number was used in calculating the nuclear ratios of the other PAN and NIC lines. In later experiments, the average nuclear number used was 2.60, an average value found in the first experiments.

Estimations of the nuclear proportions, using the conidial frequencies and average nuclear number per conidium were made according to the formula of ATWOOD and MUKAI (1955; see below).

THEORY AND NOTATION

Although a refined method for the estimation of nuclear proportions has been described (PROUT *et al.* 1953), it assumes a random distribution of nuclear types in the conidia of heterocaryons and its usefulness is limited by the observed departure from randomness in some cases (PROUT *et al.* 1953; ATWOOD and MUKAI 1955; KLEIN 1958), and by the complexity of applying it. More recently, ATWOOD and MUKAI (1955) have given a simpler formula which takes into account departures from randomness, and which gives approximately the same values as that of PROUT *et al.* Because random distribution of nuclear types in conidia is not an important consideration here, and since the application of ATWOOD and MUKAI's formula is justifiable in more cases, the latter is used as the basis of the theory stated below.

ATWOOD and MUKAI derive their formula for the estimation of nuclear proportions in conidia as follows (paraphrased): Let the frequency of the *pan, al-1* homocaryons be represented by a, that of the *nic-2, al-2* homocaryons be represented by b, and that of the heterocaryons be represented by r. Thus, $a + b + r = 1$. If N is the total number of conidia in the samples added to each plate, and if $\bar{n}$ is the average nuclear number, $N\bar{n}$ is the total nuclear population to be considered. In heterocaryotic conidia, at least one nucleus of each type is present. Thus the known number of *pan, al-1* nuclei in the population is Nr. The nuclear population in homocaryotic conidia plus those in excess of the two known in each heterocaryotic conidium is $N\bar{n} - 2Nr$. It is then assumed that in this remaining population, the ratio of the two types of nuclei is the same as that of the corresponding homocaryotic conidial types. The proportion of *pan, al-1* homocaryons among homocaryotic conidia is $a/(1-r)$. To find the number of *pan, al-1* nuclei in the total nuclear population, $N\bar{n} - 2Nr$ is multiplied by $a/1-r$ and added to Nr. This number is then divided by the total nuclear population, $N\bar{n}$, to give the approximate proportion p, of *pan, al-1* nuclei:

$$p = \frac{r(1-r) + a(\bar{n}-2r)}{\bar{n}(1-r)} \qquad \text{(I)}$$

An essential feature of this formula is the simplifying assumption that all three conidial types have equal average nuclear numbers. This is justified to some extent by the observed tendency for more homocaryotic conidia to be formed, at the expense of heterocaryotic conidia, than would be expected on the basis of random distribution of nuclei in conidia.

ATWOOD and MUKAI have not gone beyond the use of this formula in the estimation of nuclear proportions. If this formula is valid, and if the conidial frequencies found by plating represent the frequencies of conidia transferred during the selection procedure outlined above, it should be possible to predict the value of p for the next generation. Assuming *nic-2 al-2* cells to be inviable on medium containing only calcium pantothenate, the proportion of the conidial population which is able to grow is $a + r$. Because the average nuclear numbers of all types of conidia are assumed to be equal, $a + r$ also represents the proportion of the nuclear population which resides in conidia capable of growth. If the proportion of *pan, al-1* nuclei of a given heterocaryon be divided by $a + r$, one obtains the proportion of *pan, al-1* nuclei in conidia able to grow on pantothenate. If it is further assumed that equal division rates of the two nuclear types of these conidia takes place (PITTENGER and ATWOOD 1956), then this fraction will equal the frequency of *pan, al-1* nuclei in the next generation. This value, predicted from the previous generation by assuming that *nic-2, al-2* conidia cannot grow, will be denoted

$$p' = \frac{p}{a + r} \quad . \qquad \text{(II)}$$

The equivalent expression for selection in favor of the *nic-2, al-2* nuclear type is:

$$(1-p)' = \frac{(1-p)}{b+r} \quad . \tag{III}$$

If one assumes that both homocaryotic conidial types are selected against in a given transfer, and that only heterocaryotic conidia contribute to the resulting heterocaryon, one may predict the proportion of *pan, al-1* nuclei in a heterocaryon grown under these conditions. Nr represents the number of *pan, al-1* nuclei known to be present in heterocaryotic conidia; to this must be added the number of nuclei in excess of the two known (i.e., $N\bar{n}r - 2Nr$), multiplied by the proportion, $a/(1-r)$, of *pan, al-1* nuclei among them. This sum is then divided by the total number of nuclei in heterocaryotic conidia, $N\bar{n}r$, to obtain the proportion of *pan, al-1* nuclei. Thus the proportion of *pan, al-1* nuclei in heterocaryotic conidia will be:

$$p'' = \frac{Nr + \frac{a(N\bar{n}r - 2Nr)}{1-r}}{N\bar{n}r} \tag{IV}$$

$$= \frac{(1-r) + a(\bar{n}-2)}{\bar{n}(1-r)} \quad , \tag{V}$$

and p'' will represent the predicted frequency of *pan, al-1* nuclei of a heterocaryon derived from heterocaryotic conidia only. The predicted frequency, $(1-p)''$, of *nic-2, al-2* nuclei under the same conditions will be:

$$(1-p)'' = \frac{(1-r) + b(\bar{n}-2)}{n(1-r)} \quad . \tag{VI}$$

It may be seen that as long as the average number of nuclei per conidium is nearly two, the last two formulae will approximate 0.50. This, however, is somewhat unrealistic when it is realized that the value of $\bar{n}$ for heterocaryotic conidia will in all probability be greater than 2, this being the lower limit of the nuclear frequency distribution for this conidial type. A correction of this feature, made by disregarding uninucleate conidia in computing $\bar{n}$ would modify it by only ten percent in most cases. The effect on the values of p and $1-p$ is almost negligible for the present analysis. Now, as $\bar{n}$ becomes larger, the values p'' and $(1-p)''$ of selected lines in which only heterocaryotic conidia are able to grow will tend toward 0.50. The only stable value is 0.50, theoretically, since the two formulae (V and VI) are dominated by the 1:1 ratio of the two nuclear types essential to every heterocaryotic conidium.

A general formula may now be derived, from which variable contributions of one homocaryotic conidial class selected for may be found, assuming the other homocaryotic class to be completely selected against.

If $N\bar{n}a$ is the total number of nuclei in conidia homocaryotic for *pan, al-1*, and if x is taken to be the proportion of these nuclei which contribute to the next generation, then $N\bar{n}ax$ is the number of these nuclei which contribute to the next

generation. By adding the quantity $N\bar{n}ax$ to both numerator and denominator of the right hand side of Formula IV, we obtain, after simplifying:

$$p_{t+1} = \frac{r(1-r) + a(\bar{n}r-2r) + \bar{n}a(1-r)x}{\bar{n}(1-r)\ (r+ax)} \quad , \qquad \text{(VII)}$$

where t is the generation from which the values of a, r, and $\bar{n}$ are taken, and where p_{t+1} is the value of p in the next. This expression may be used to find the proportion of *pan, al-1* homocaryons which contribute to the latter generation, relative to the heterocaryotic type, by solving for x. The proportion of the heterocaryotic type which contributes is assumed to be 1.00. The formula may be modified to describe the relative contribution of *nic-2, al-2* homocaryons if *pan, al-1* homocaryons are absolutely selected against, as follows:

$$(1-p)_{t+1} = \frac{r(1-r) + b(\bar{n}r-2r) + \bar{n}b(1-r)y}{\bar{n}(1-r)\ (r+by)} \qquad \text{(VIII)}$$

where y is the proportion of *nic-2, al-2* conidia that grow, or where y is the extent to which all *nic-2, al-2* conidia contribute to the final nuclear population.

Now, if $x = 0$, formula VII reduces to the value of p'', and if $x = 1$, formula VII reduces to that of p'. If the actual value of p found in a given generation is used as p_{t+1}, the magnitude of x, the selective value of the homocaryotic conidial class, may be obtained.

To summarize: Given certain conditions, it is possible to predict the frequencies of nuclei in a heterocaryon by analyzing the conidial population from which the heterocaryon is derived. The predicted frequencies may be obtained by assuming that *pan, al-1* homocaryons and heterocaryons grow and contribute equally to the resulting heterocaryon (prediction p'), that *nic-2, al-2* homocaryons and heterocaryons grow equally well (prediction $(1-p)'$), or that only heterocaryons grow (predictions p'' and $(1-p)''$). These predictions may be compared graphically to the observed value of p or $(1-p)$ of the heterocaryons at each generation during selection. Furthermore, inequalities in the relative contribution, or selective value, of the heterocaryotic and one homocaryotic conidial class may be found by analyzing the data with Formulae VII and VIII.

RESULTS

Reliability of methods

Two *pan, al-1* + *nic-2, al-2* heterocaryons were analyzed in quadruplicate in order to estimate the range of variation in the nuclear ratio determinations. From each heterocaryon, four platings were made to determine the frequencies of the three conidial types, and four samples of conidia were stained for the determination of average nuclear numbers. The value of p for the first heterocaryon ranged from 0.92–0.95 (std. dev. 0.013), and for the second, from 0.70–0.74 (std. dev. 0.016). The data indicate that values of p are consistent within about 0.03 (two std. dev's.).

Selection on mixtures of homocaryotic conidia

Since homocaryotic conidia on unsupplemented medium can develop short germ tubes, heterocaryosis between conidia selected for and those selected against might be expected to occur by the anastomosis of these germ tubes. To test the extent of this possible heterocaryosis, 0.1 ml aliquots of a 1:1 mixture of *pan, al-1* and *nic-2, al-2* conidia containing 10^5 conidia per ml were placed on slants of standard minimal medium, minimal + pantothenate, and minimal + nicotinic acid. This duplicated as nearly as possible the conditions prevailing during the selection experiments with regard to media and the distribution of conidia on the slants.

In the tubes of minimal medium, no growth took place. Conidia from the mycelia developing in the supplemented tubes were plated on minimal and both singly supplemented plating media to test for the presence of the nuclear type selected against. No nuclei of these types expressed themselves in platings of 5×10^4 conidia. It may be concluded that in these conditions, heterocaryotic associations involving conidia selected against do not occur to any significant extent.

Changes of nuclear proportions during selection

The changes in nuclear proportions during selection were gradual. In the PAN lines, where *nic-2, al-2* homocaryons were selected against, the frequency, p, of the *pan, al-1* nuclear type increased from about 0.70 to about 0.95 or more in eight transfers. Selection was maintained further in two of these lines, PAN-1 and PAN-4. *Nic-2, al-2* nuclei disappeared from PAN-1 five transfers later, and still remained in PAN-4 ten transfers later, at which point the experiment was terminated. The data are shown in Table 1 and in Figure 1.

The frequencies expected on the basis of p' and p'' are also shown in Table 1 and in Figure 1. In the figure, the points on the broken or dotted lines are the predicted values for points at the same transfer on the "observed" (solid) curve. The predicted values have been connected to reveal more clearly any consistent deviations from the observed values. Statistical comparison of single observed and expected values was deemed unsuitable because of the unknown extent of the variation of individual estimates of p.

The points on the p'' curve show a large and consistent deviation from those observed. The assumption basic to this prediction, namely that only heterocaryotic conidia grow, clearly does not describe the course of selection. In the case of the p' prediction, the points agree very well with those observed. However, the agreement is meaningful only before the frequency of *pan, al-1* nuclei reaches 0.95. Above this range, the quantification of the minority homocaryotic conidial type becomes increasingly difficult, and the variation in nuclear proportions from generation to generation becomes very slight. Thus the frequencies of *pan, al-1* nuclei in the PAN lines, before they reach the value of 0.95, may be compared to the prediction p' by pooling the data for all four lines, and estimating (1) the average value of the differences of p and p', and (2) the standard deviation of this difference. The average difference, that is, the sum of the differences $(p-p')$

TABLE 1

PAN lines: observed nuclear frequencies (p) *and those predicted* (p′ *and* p″) *during the course of selection*

Transfer	PAN-1 p	p′	p″
0	.66	..	..
1	.80	.81	.56
2	.86	.86	.59
3	.88	.89	.51
4	.91	.91	.54
5	.95	.94	.55
6	.96	.95	.64
7	.96	.96	.68
8	.99	.97	.58
9	.99	.99	.52
10	.98	.99	.59
14	.996	..	..
15	1.00	..	..

Transfer	PAN-2 p	p′	p″
0	.75	..	..
1	.87	.81	.56
2	.84	.87	.68
3	.90	.89	.51
4	.92	.93	.54
5	.94	.93	.56
6	.90	.96	.62
7	.96	.95	.66
8	.95	.97	.58

Transfer	PAN-3 p	p′	p″
0	.72	..	..
1	.86	.78	.62
2	.86	.87	.59
3	.89	.90	.51
4	.90	.92	.54
5	.87	.92	.56
6	.92	.90	.61
7	.94	.93	.67
8	.98	.95	.58

Transfer	PAN-4 p	p′	p″
0	.76	..	..
1	.83	.82	.58
2	.88	.85	.64
3	.91	.89	.51
4	.96	.91	.54
5	.95	.96	.56
6	.97	.98	.61
7	.98	.98	.68
8	.98	.98	.58
9	.98	.99	.52
10	.98	.98	.59
14	.994	..	..
16	.9994	..	..
17	.9994	..	..
18	.9999	..	..
19	.9994	..	..

divided by the 21 comparisons made, is + 0.0024. This indicates that the values of p conform very well to the prediction p', and that the deviations are not consistent in their direction. The standard deviation of the difference, using 0.00 as the average, is 0.032; this value denotes the general magnitude of the differences of p and p' and is not significantly different from the range of variations found in the replicate platings of individual heterocaryons. The simple assumptions involved in the p' prediction, therefore, may be used to describe the course of selection in the PAN lines.

In all four of the NIC lines, in which *pan, al-1* homocaryons were absolutely selected against, the frequency of the *nic-2, al-2* nuclei, $1-p$, increased from as low as 0.42 to 0.50–0.58, in which range it remained in subsequent transfers with

no consistent departures. The data are given in Table 2 and in Figure 2, together with the $(1-p)'$ and $(1-p)''$ predictions.

The relationship of the observed values of $1-p$ to the two predictions in the

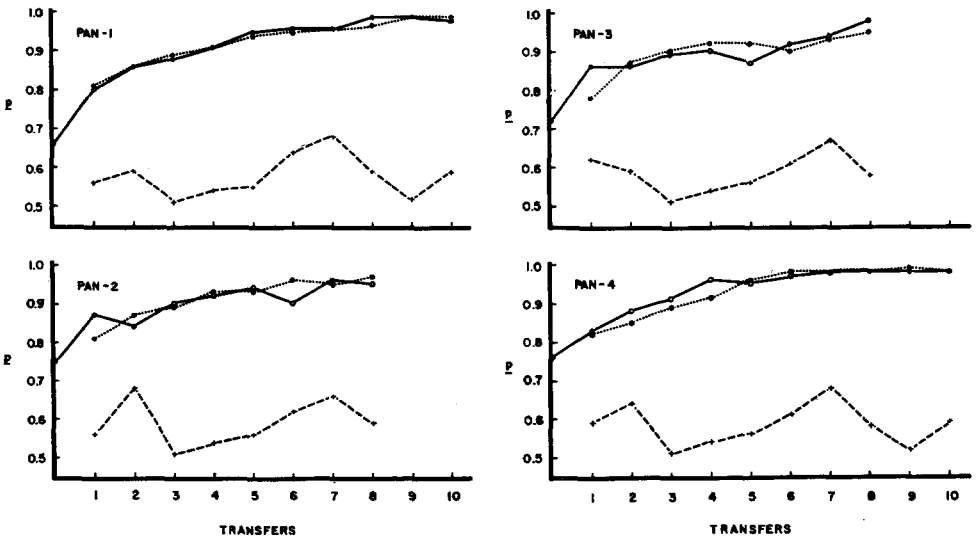

FIGURE 1.—Changes in the observed (p) and predicted (p' and p'') frequencies of *pan, al-1* nuclei taking place in the PAN lines during the course of selection. Key: p, solid line; p', dotted line; p'', dashed line.

TABLE 2

NIC lines: observed nuclear frequencies (1–p), and those predicted ((1–p)' and (1–p)") during the course of selection

Transfer	NIC-1 1–p	(1–p)'	(1–p)"	Transfer	NIC-2 1–p	(1–p)'	(1–p)"
0	.47	..	..	0	.42	..	..
1	.50	.71	.49	1	.49	.75	.49
2	.54	.72	.50	2	.57	.71	.50
3	.58	.70	.51	3	.58	.71	.53
4	.58	.75	.52	4	.53	.70	.52
5	.58	.77	.53	5	.52	.72	.51

Transfer	NIC-3 1–p	(1–p)'	(1–p)"	Transfer	NIC-4 1–p	(1–p)'	(1–p)"
0	.46	..	..	0	.53	..	..
1	.52	.72	.49	1	.48	.77	.49
2	.54	.72	.50	2	.51	.74	.54
3	.53	.73	.52	3	.54	.72	.50
4	.55	.72	.51	4	.57	.76	.51
5	.50	.70	.52	5	.57	.74	.53

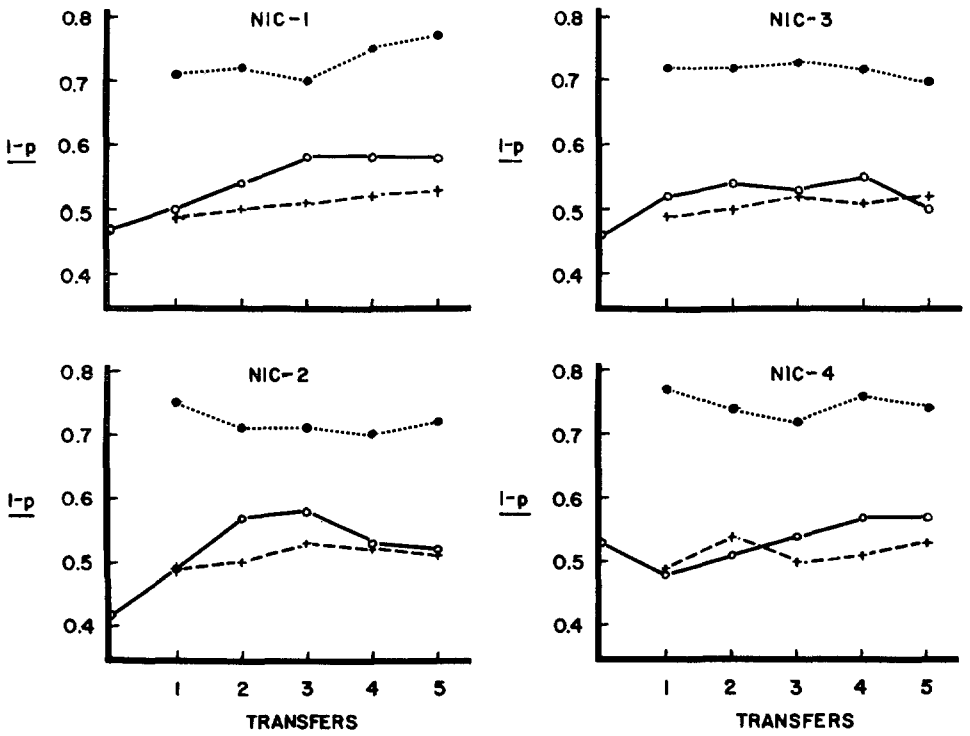

FIGURE 2.—Changes in the observed $(1-p)$ and predicted ($(1-p)'$ and $(1-p)''$) frequencies of *nic-2, al-2* nuclei taking place in the NIC lines during the course of selection. Key $1-p$, solid line, $(1-p)'$, dotted line; $(1-p)''$, dashed line.

NIC lines is quite different from that in the PAN lines. In the NIC lines, $1-p$ deviates widely from the prediction $(1-p)'$, and tends to follow the prediction $(1-p)''$ instead. By comparing $1-p$ to $(1-p)''$ in a manner similar to that used in the case of the PAN lines, the average difference between $1-p$ and $(1-p)''$ is $+ 0.029$. This indicates that the observed values lay, on the average, consistently 0.03 above those predicted according to the assumption that only heterocaryotic conidia grow. The standard deviation of the difference, using 0.03 as the average, is 0.03, a figure compatible with the range of variation found in platings of individual heterocaryons.

Because the only stable value of $(1-p)''$, theoretically, is 0.50, and because the value of $1-p$ appears to be relatively stable at approximately 0.54, it is tentatively concluded that in the NIC lines, conidia homocaryotic for *nic-2, al-2* nuclei are contributing slightly to the final nuclear population at each transfer. In light of the fact that 0.54 is the average value of $1-p$ for the NIC lines throughout the experiments, it is possible to estimate the proportion of *nic-2, al-2* homocaryons, γ, contributing to each generation. This is done by choosing the values of a, b, r, $\overline{n}$, and $(1+p)_{t+1}$ for specific transfers, substituting these in Formula VIII, and solving for γ. In Table 3 are presented the results of such an analysis for three

TABLE 3

Representative values of y: *Data for generation 2 of the NIC lines 1, 3, and 4, with observed and expected values of 1–*p *for generation 3, and calculated values of* y*

Line	Transfer	r	a	b	$\bar{n}$	$(1-p)_2$	$(1-p)''_3$ Expec.	$(1-p)_3$ Observ.	γ
NIC–3	2 to 3	.414	.259	.327	2.64	.54	.52	.53	0.04
NIC–4	2 to 3	.414	.292	.294	2.64	.51	.50	.54	0.14
NIC–1	2 to 3	.482	.228	.290	2.64	.54	.51	.58	0.24

* r, a, and b are the frequencies of heterocaryons, *pan, al–1* homocaryons and *nic–2, al–2* homocaryons, respectively. $\bar{n}$ is the average number of nuclei per conidium. See text for further explanation.

lines in the transfer from the second to the third generation. The transfers are chosen according to the differences between the observed and the expected values at the third generation. The first pair of values, differing by 0.01, gives $\gamma = 0.04$, the second, differing by 0.04, gives $\gamma = 0.14$, and the third, differing by 0.07, gives $\gamma = 0.24$. To represent the general behavior exhibited by the NIC lines, the data of the third case listed may be taken, with the provision that $(1-p)_3$ equal 0.54. The latter value, the average of $1-p$ throughout, is 0.03 above the $(1-p)''$ prediction. The value of γ using this value of $1-p$ is 0.09, which is a rough approximation of the extent to which *nic-2, al-2* conidia contribute to each generation if $1-p$ is maintained at an equilibrium value of 0.54.

To summarize: (1) In the PAN lines, the selective value of *pan, al-1* conidia, x, is 1.00, relative to the heterocaryotic conidial type. This characteristic leads to the reduction in frequency and the virtual disappearance of the *nic-2, al-2* nuclear type after 15 to 20 transfers. (2) In the NIC lines, the heterocaryotic conidia appear to be the main contributors to each generation, and the selective advantage of the *nic-2, al-2* conidia is approximately 0.09, relative to that of the heterocaryotic conidia. This leads to the establishment of an equilibrium of the nuclear proportions which favors the *nic-2, al-2* nuclear type slightly.

The selective disadvantage of nic-2, al-2

To disclose more clearly the apparent selective disadvantage of *nic-2, al-2* conidia as compared to the heterocaryotic conidia in the NIC lines, homocaryotic cultures of *nic-2, al-2* and *pan, al-1* were isolated during the selection experiments. These were compared to the heterocaryon and to each other in various ways.

1. The growth rates of *pan, al-1* on pantothenate supplemented medium, of *nic-2, al-2* on nicotinic supplemented medium, and of the heterocaryon on both types of media were found to be indistinguishable, by the tube method of determining mycelial growth rates (RYAN, BEADLE and TATUM 1943). In a second experiment, the nuclear ratios of three heterocaryons at the beginning and end of 450 mm growth tubes were determined. In no case was the difference in the value of p more than 0.03 at the beginning than at the end. This result indicated that the rates of increase of the nuclear types were essentially equal during the growth of these heterocaryons (PITTENGER and ATWOOD 1956), and that the

disadvantage of *nic-2, al-2* in the NIC lines could not be explained in terms of nuclear competition in growing heterocaryotic mycelia.

2. The early development of mycelia from conidial inocula was then studied. Slants of standard minimal and supplemented media, comparable to those used in the selection experiments, were inoculated with 0.1 ml of conidial suspension of *pan, al-1, nic-2, al-2*, or a heterocaryon. After 40 hours, the growth in tubes inoculated with *nic-2, al-2* conidia in numbers similar to those used in transferring the NIC and PAN lines was just visible as a thin network of hypae covering the slant. After the same interval, inocula of the same numbers of conidia of both *pan, al-1* and the heterocaryon had given rise to rich mycelia with aerial mycelium. This result is consistent with the indication from experiments with the NIC lines that the growth of heterocaryotic conidia swamps that of *nic-2, al-2* homocaryons, whereas in the PAN lines, *pan, al-1* and heterocaryotic conidia contribute equally to the next generation.

This same method was used to test the effects of changes in the medium upon the establishment of mycelia. In a second series of experiments, the early growth of the heterocaryon and of *nic-2, al-2* from conidial inocula was compared on combinations of one or two percent sucrose and nicotinic acid or nicotinamide. The medium used in the selection experiments, containing one percent sucrose and nicotinic acid, was found to be least favorable for the growth of *nic-2, al-2*. Growth proceeded somewhat faster on two percent sucrose and nicotinic acid, and the substitution of nicotinamide for nicotinic acid resulted in a developmental rate comparable to that of the heterocaryon. The growth of the heterocaryon did not differ significantly with these substitutions in the medium. The differential effect of nicotinic acid and nicotinamide has been mentioned by BARRATT *et al.* (1954). This information will be utilized in the further selection experiments reported below.

3. From the preceding experiments, there appears to be no competition between *pan, al-1* and *nic-2, al-2* nuclei when they are associated in heterocaryons, but the early growth of *nic-2, al-2* conidial inocula is retarded, compared to that of other inocula, on the medium used in the selection experiments. It is postulated then, that selection against *nic-2, al-2* nuclei in the NIC lines operates before the fusion of germ tubes and mycelium developing from *nic-2, al-2* and heterocaryotic conidia. A more direct test of this assumption was made in the following experiment.

It has been shown that the early development of mycelia derived from *pan, al-1* and heterocaryotic conidia takes place at approximately the same rate. By inoculating slants containing both pantothenate and nicotinic acid with a 1:1 mixture of *pan, al-1* and *nic-2, al-2* conidia, and determining the nuclear ratio of the resulting heterocaryon, the extent to which *nic-2, al-2* nuclei are selected against may be found. Because the conidia of *pan, al-1* and *nic-2, al-2* are in a 1:1 ratio in the present experiment, immediate fusion of their germ tubes would be expected to result in equal proportions of the two nuclear types in the heterocaryon. As the concentration of the 1:1 mixture is increased, it is expected that a

1:1 nuclear ratio in the resulting heterocaryon will be approached. As fewer conidia are used in the inoculum, more growth may occur before fusion, and selection would be expected to operate to a greater extent.

The result of this experiment was that when 10^6 conidia were used, $1-p = 0.47$; 10^5 conidia: $1-p = 0.54$; 10^3 conidia: $1-p = 0.29$; 10^2 conidia: $1-p = 0.15$ (the 10^4 tube was lost). These nuclear ratios indicate that selection against *nic-2, al-2* becomes more pronounced as the concentration of conidia decreases. It is concluded that selection against *nic-2, al-2* in the NIC lines operates before the fusion of growth from conidia used in the transfers.

Further selection experiments

The following brief selection experiments were made to check the previous findings, to test the effect of different initial proportions, and to test the effect of different media, all with a view to testing further the hypotheses presented above.

1) A heterocaryon constituted of 0.21 *pan, al-1* and 0.79 *nic-2, al-2* nuclei was serially transferred on pantothenate supplemented medium for six generations. The value of p rose to 0.97 in six transfers, and was reasonably well in accord with the p' prediction (Figure 3a).

2) A heterocaryon containing 0.80 *nic-2, al-2* nuclei was transferred on nicotinic acid supplemented medium for seven generations. The proportion of *nic-2, al-2* nuclei declined within three generations to 0.51, and followed the $(1-p)''$ prediction rather closely. After these three transfers, the medium was changed to contain two, instead of one percent sucrose. From this time the observed curve of $1-p$ consistently rose, departing from the $(1-p)''$ prediction, and followed a middle course between $(1-p)'$ and $(1-p)''$. The data are shown graphically in Figure 3b. The difference in the behavior of the heterocaryon on the two levels of sucrose may be understood in terms of the beneficial effect of added sucrose on the rate of mycelial development of *nic-2, al-2*.

3) A heterocaryon containing 0.55 *nic-2, al-2* nuclei was transferred for four transfers on nicotinamide supplemented medium. Here, the $1-p$ curve follows the $(1-p)'$ prediction (Figure 3c), and the final proportion of *nic-2, al-2* nuclei was 0.92. Nicotinamide appears to equalize the selective advantage of *nic-2, al-2* and heterocaryotic conidia, a finding also indicated by the effect of nicotinamide on the rate of mycelial establishment of *nic-2, al-2*.

4) A heterocaryon containing 0.17 *pan, al-1* nuclei was transferred for seven generations on minimal medium. In this case, the p curve follows p'' quite closely at first, but then tends to fluctuate slightly above 0.50 (Figure 3d). The p values average 0.023 above the p'' prediction. The discrepancy may represent a slight differential mortality of *nic-2, al-2* conidia over *pan, al-1* in plating for nuclear ratio, but the experiment indicates that p will stabilize around 0.50 if heterocaryotic conidia are the sole contributors to each generation (See Theory and Notation).

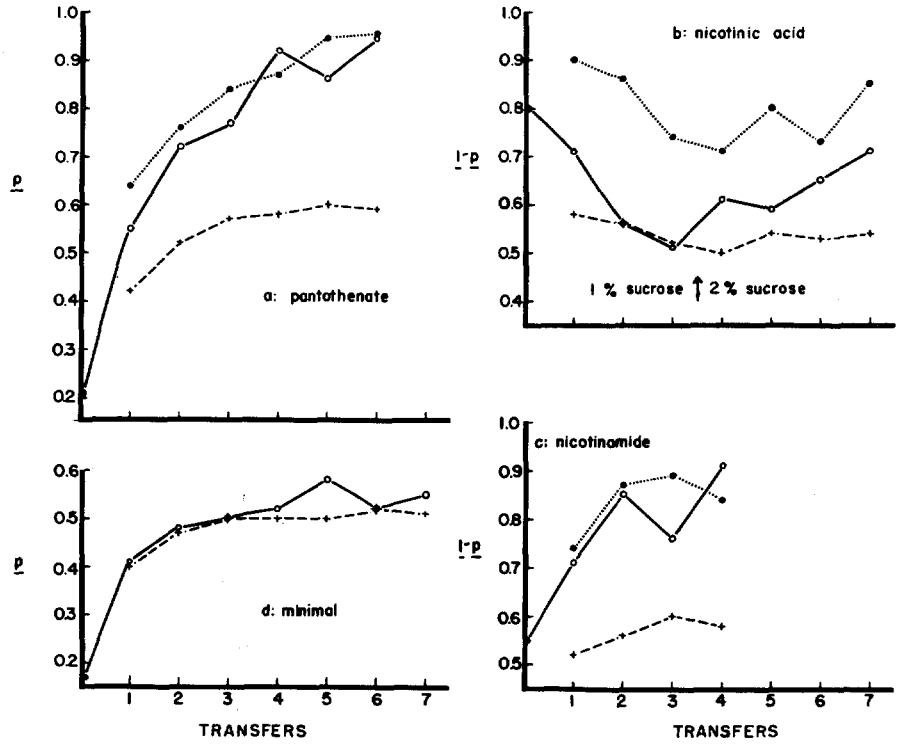

FIGURE 3.—Changes in nuclear frequencies taking place during selection of heterocaryons on different media. Key: p or $1—p$, solid lines; p' or $(1—p)'$, dotted lines; p'' or $(1—p)''$, dashed line.

DISCUSSION AND CONCLUSIONS

The foregoing experiments illustrate quantitatively the dampening effect of heterocaryosis on selection against "disadvantageous" nuclear types. In the PAN lines, where the *nic-2, al-2* nuclear type was selected against, the loss of *nic-2, al-2* nuclei was rapid when it was present in high frequency, but may be maintained at low frequencies for considerable periods of time. The latter feature permits the heterocaryon to adapt readily to a reversal of nutritional conditions. In the NIC lines, where nicotinic acid is the supplement in the medium, both *pan, al-1* and *nic-2, al-2* nuclei are maintained in almost equal frequency. Here, under conditions where *nic-2, al-2* and heterocaryotic mycelia may grow at the same rate, *nic-2, al-2* homocaryons appear to be selected against almost as strongly as *pan, al-1* homocaryons, and heterocaryotic conidia are the main contributors to each generation. The slow rate of germination of *nic-2, al-2* homocaryons, as compared to the heterocaryons, appears to be a sufficient explanation of this finding.

The model which has been proposed has been tested by comparing predictions, based on simple assumptions, with the observed changes in nuclear proportions.

The predictions p' and $(1-p)'$ are based on the assumptions a) that conidia of the homocaryotic class selected for and of the heterocaryotic class develop at the same rate, b) that the two nuclear components of heterocaryotic mycelia divide at the same rate, c) that each conidial type have the same average nuclear number, and d) that there be no differential mortality of conidial types in testing for nuclear ratio that does not also prevail in the transfer procedure. The basis of the predictions p'' and $(1-p)''$ differ only in the first assumption, which is in this case modified to state that only heterocaryotic conidia develop.

The first assumption and its alternate may be modified quantitatively in the light of experimental data, as seen in the case of the NIC lines. The second assumption finds support in the line transferred on minimal medium (Figure 3d) and in the observed stability of nuclear proportions during mycelial growth. The third point, (c), is open to some question, being only partially justified (ATWOOD and MUKAI 1955). It is, however, a simplifying assumption which does not do much violence to the accuracy of the estimations and predictions. A modification of the model based upon the assumption of random association of nuclei in conidia, and the concomitant inequalities in nuclear number of the three conidial types would not affect the interpretations appreciably, except in the case of highly disparate ratios. All data are reasonably consistent with the last assumption, although no direct proof of it has been obtained.

The data, therefore, may be analyzed in terms of the model quite well. There is room for minor changes in the model, based upon more refined data, but its present form is the simplest that may be derived from the present knowledge of the system.

In the context of the experiments, in which one homocaryotic conidial type is absolutely selected against, the model allows one to determine the selective value (x or y) of the other homocaryotic conidial type in relation to the heterocaryotic type. This is a valid calculation only if it is known that the nuclei involved in heterocaryotic conidia and mycelia have in general the same division rates. Thus, when the homocaryotic conidial class selected for has a selective value equal to or greater than 1.00 (relative to the heterocaryotic type), the frequency of the corresponding nuclear type may reach the value of 1.00. However, if its selective value is anything less than 1.00, both types of nuclei will be maintained in a large population permanently under constant nutritional conditions. The equilibrium nuclear proportions are dictated by the relative selective value of the two viable conidial types. This is an inescapable feature of Formulae VII and VIII.

The formulae presented may be modified to conform to other selective conditions. For instance, if the heterocaryotic and one homocaryotic conidial types have equal selective values, and the third conidial type has a selective value between 0 and 1.00, the right hand sides of Formulae II and III may be changed to $\frac{p}{a+r+yb}$ and $\frac{1-p}{b+r+xa}$, respectively. Also, if both homocaryotic conidial types

have selective values less than that of the heterocaryotic type, Formulae V and VI may be expanded to take this into account.

Whether the model proposed here may be applied to similar patterns of selection in nature remains to be determined. The strains of *N. crassa* used here, although not representative of naturally occurring ones in their genetic heterogeneity, do illustrate the fate of nuclei bearing certain genes which are "recessive" in the sense of BEADLE and COONRADT (1944). The model would have to be elaborated further to take into account such factors as incomplete recessiveness and differences in the division rates of different nuclear types in heterocaryons.

A last point is the comparison of nuclear selection in *N. crassa* and the theoretical pattern of selection against a lethal gene in diploid populations. The two types of organisms are exactly comparable in the following particulars:

1) A "deleterious" element (gene or nucleus) of the population may have little effect when associated with an advantageous element, but may be lethal when not so associated.

2) The reduction in frequency of a deleterious element may be referred to the lack of fitness of the recessive homozygotes in one case, and of disadvantageous homocaryons in the other.

3) As the frequency of the deleterious element decreases, a greater proportion of them are associated with the advantageous element. The rate of disappearance of the deleterious element therefore becomes slower and slower with time.

4) If the "deleterious" element should, through altered environmental circumstances, come to have a greater selective value than its alternate, selection may restore the former to prominence rather quickly. The maintenance of variability is therefore of considerable significance in unstable environments.

The systems are different in these respects: In diploids, sexual reproduction is a process by which combinations of genes are made; these combinations are made in pairs. In *N. crassa*, asexual reproduction of a heterocaryon involves the association of nuclei. The number of nuclei in each conidium may vary from one to ten or more.

With these points in mind, one may graphically compare the process of selection in the two types of organisms. In Figure 4, the frequency of the "lethal" gene or nucleus is plotted against the number of generations of selection. The points on the curve of the disappearance of the recessive lethal gene in diploids are calculated from the simple formula: $q_n = q_o/(1 + nq_o)$ (DOBZHANSKY 1953), where q_o is the initial frequency of the lethal (0.34 in the present example), and n the generations of selection. The curve for *N. crassa* nuclear proportions is that of the PAN-1 line; q in this case represents the frequency of the *nic-2*, *al-2* nucleus, starting at 0.34.

It may be seen that the maintenance of recessive lethals in diploids is more efficient than maintenance of "lethal-bearing" nuclei in *N. crassa*. The level of the lethal nucleus in *N. crassa* drops below 0.01 between the tenth and fifteenth generations, whereas this level is reached by lethals in large diploid populations

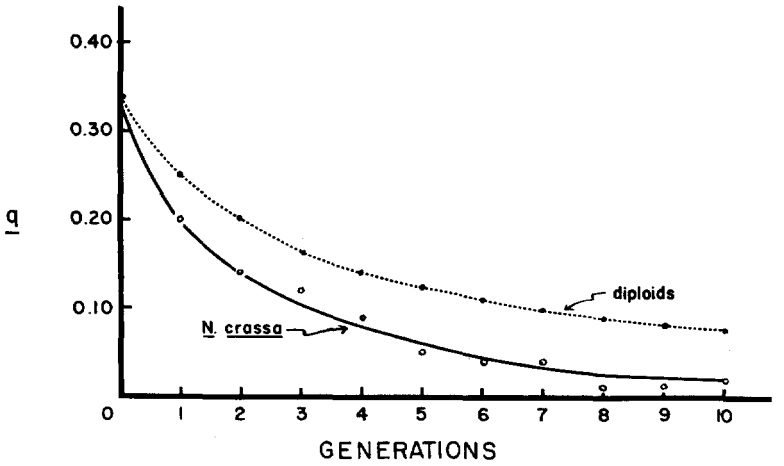

FIGURE 4.—Graphic representation of the decrease in frequency (q) of a "lethal" nucleus over ten asexual transfers of a heterocaryon of *N. crassa* or of a lethal gene during ten generations of random interbreeding of a diploid population.

only by the ninety-eighth if $q_0 = 0.34$. The similarity in the pattern of change, however, is attested to by the general shape of the two curves.

SUMMARY

1. Conidial transfers of heterocaryons of biochemically deficient nuclear types of *N. crassa* have been made serially on media on which one of the homocaryotic conidial types could not propagate. The changes in the nuclear proportions over many generations have been observed, and compared to the changes expected on the basis of the conidial frequencies of the transfer inocula.

2. From known factors of the system, an algebraic model has been proposed to account for the observed changes in nuclear proportions during selection. This model includes a method for determining the selective values of conidial types under different nutritional conditions.

3. It has been found that nuclear types absolutely selected against may be maintained in a nuclear population for a considerable number of transfers by the association with another type in heterocaryotic conidia. This allows a heterocaryon to adapt quickly to changes of nutritional conditions.

4. The pattern of selection operating during the asexual reproduction of *N. crassa* heterocaryons has been compared to that operating during the sexual reproduction of diploids.

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