NOTES AND COMMENTS

MONOKARYON FREQUENCY AMONG SURVIVORS FROM DIKARYOTIC MYCELIAL FRAGMENTS

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SUMMARY

If unicellular fragments of dikaryotic fungal mycelia are treated with environmental agents which inactivate their nuclei, a sharp rise of monokaryotic fragments among the survivors is expected. Mathematical evidence is presented that the same is also true for homogeneous multicellular fragment populations. It is shown that almost the same increase in monokaryon frequency is to be expected if the fragment population is heterogeneous and composed of different size fragments, regardless of whether these sizes are randomly or non-randomly distributed in the population. The curves for monokaryon frequencies among survivors are almost identical for all four situations considered. We may conclude that dikaryotic mycelia of higher fungi, such as *Schizophyllum commune*, are very suitable systems for investigating the effect of environmental agents on the nuclei.

In many basidiomycetous fungi, like *Schizophyllum commune*, the dikaryotic stage consists of binucleate cells. The number of nuclei is exactly two in all cells, and in the heterothallic species one nucleus is derived from each of the monokaryons (Raper, 1966). Assuming a population of unicellular mycelial fragments of a dikaryon, we expect that killing agents such as ultraviolet irradiation, X-rays or fungicides which inactivate the nuclei, will act upon them independently. Many of the surviving fragments will thus become monokaryotic, and the frequency of these will rise quickly as the survival frequency decreases.

If all the fragments are unicellular, and $0 < p < 1$ ($q = 1 - p$) is the probability that each nucleus will be inactivated, the binomial distribution for two trials (corresponding to two nuclei per fragment) gives the probability of fragments in which (a) both nuclei are killed as p^2 , (b) only one of the nuclei is killed (= monokaryons) as $2pq$, and (c) dikaryotic fragments as q^2 . The conditional probability, P_s , of monokaryons among survivors is thus $\frac{2pq}{1 - p^2}$. We shall generally denote by n the number of cells per fragment, and $2n$ the number of nuclei per fragment. In practice, however, the mycelial fragments are achieved by mechanical maceration of the intact

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dikaryotic mycelium, and thus the number of cells per fragment (*fragment size*) varies and can be estimated only with the greatest difficulty (Parag and Giami, 1975).

If, as a result of maceration, we produce a *homogeneous fragment population*, in which all the fragments are of the same size, we can show that the number of cells per fragment has no effect on the curve representing the frequency of monokaryons as a function of the survival proportion. In fact, the frequency of monokaryons among survivors is the same, regardless of fragment size. If monokaryons of two mating types, A1 and A2 exist, each cell in the dikaryon resulting from the mating will be of type A1+A2. Given that at least one nucleus is not inactivated, the probability of any fragment being reduced to a monokaryon is equal to the probability of all A1 nuclei being killed and one or more A2 nuclei surviving, plus the probability of all A2 nuclei being killed with one or more A1 nuclei surviving, that is

$$2p^n(1-p^n).$$

Therefore, the probability of monokaryons is

$$P_m = \frac{2p^n(1-p^n)}{1-p^{2n}}. \quad (1)$$

In the homogeneous population when $n > 1$, the survival rate is

$$P_s = 1 - p^{2n} \text{ so that } p = \sqrt[n]{1 - P_s}.$$

Substituting this last value for p , we have that

$$P_m = \frac{2\sqrt{1-P_s}(1-\sqrt{1-P_s})}{1-(1-P_s)} = \frac{2\sqrt{1-P_s}-2+2P_s}{P_s}, \quad (2)$$

which is independent of the value of n .

We thus see that the value n of the numbers of cells per fragment does not affect the probability of monokaryons among the survivors, provided all the dikaryotic fragments have the same number of cells. In practice, there may be a mixture of fragments with different numbers of cells (*heterogeneous population*).

In order to test the dependence of monokaryon frequency on survival in heterogeneous populations, we simulated experimental conditions by constructing theoretical curves for two types of heterogeneous cell populations and compared these with the theoretical curve for the unicellular population (fig. 1). If $\bar{n}$ is the mean number of cells in a macerated mycelium, we should expect the number of cells per fragment to follow a Poisson distribution with this mean. From (1), we see that the general formula for the probability of monokaryons would be

$$P_m = e^{-\bar{n}} \left[\bar{n} \cdot \frac{2p(1-p)}{1-p^2} + \frac{\bar{n}^2}{2!} \cdot \frac{2p^2(1-p^2)}{1-p^4} + \frac{\bar{n}^3}{3!} \cdot \frac{2p^3(1-p^3)}{1-p^6} + \dots \right]. \quad (3)$$

Similarly, the formula for the probability, P_s , of survival would be

$$P_s = e^{-\bar{n}} \left[\bar{n}(1-p^2) + \frac{\bar{n}^2}{2!} \cdot (1-p^4) + \frac{\bar{n}^3}{3!} (1-p^6) + \dots \right]. \quad (4)$$

Values for $\bar{n} = 3$ and $\bar{n} = 10$ were tried on the assumption that the real experimental mean was within this range. From fig. 1 we can see that the curves for these means nearly coincide with the curve for $n = 1$; only the doses for which identical survival frequencies are achieved are highly different. This can be seen from the different values p_1, p_3, p_{10} corresponding to identical survival frequencies in fig. 1.

The slight reduction in the values achieved with heterogeneous populations compared with the theoretical is not accidental. The reason is that the survival ratio and the monokaryon ratio in a heterogeneous population are combined values of all the separate classes. For example, if we mix unicellular fragments with ten-cellular fragments in the proportions 1 : 3, we find that

$$\begin{aligned} P_{s10} &= 0.8784; & 0.8784 \times 0.75 &= 0.6590 & P_{m10} \times 0.75 &= 0.3885 \\ P_{s1} &= 0.1900; & 0.1900 \times 0.25 &= 0.0475 & P_{m1} \times 0.25 &= 0.2375 \\ & & P_s &= 0.7065 & P_m &= 0.6250. \end{aligned}$$

In the homogeneous population, a value of $P_s = 0.7065$ should give $P_m = 0.715$. This deviation, resulting from heterogeneity much more extreme than would be expected in experimental conditions, is not very great. If we take extreme conditions, in which we have a population composed of unicellular and ten-cellular fragments, and for each p we take the proportion which gives the largest deviation (fig. 1), we note that for all but high values of P_s (> 0.5) the deviation is extremely small and would probably be insignificant in relation to experimental error.

Efforts had been made to use heterokaryotic spores of *Neurospora* (Atwood, 1954; Atwood and Mukai, 1954) and of *Phycomyces* (Cerdá-Olmedo and Reau, 1970) for the distinction between nuclear inactivation (Atwood's term, or mitotic lethality, Cerdá-Olmedo and Reau's term) and other types of lethal effects of environmental agents. In these heterokaryons, the shape of the curve representing monokaryon frequency among survivors depends on the number of nuclei per spore, the distribution of number of nuclei in the spores, and the nuclear ratio of the two types of nuclei in the spores (see also Atwood and Mukai, 1954). In *Neurospora*, all three parameters are variable (Prout *et al.*, 1953; Atwood and Mukai, 1954, 1955). In *Phycomyces* the number of nuclei per spore is 1-6, and the data of Cerdá-Olmedo and Reau (1970) show significant deviation from random distribution of numbers of nuclei in the spores.

In dikaryotic systems, on the other hand, the number of nuclei per cell is exactly two, and the two genotypes are distributed exactly one to one in each cell. The variable parameters, namely the fragment sizes and the distribution of these sizes, have been shown in this paper to have a very small or negligible effect on the expected frequency of monokaryons among survivors. This makes dikaryotic mycelia of higher fungi a suitable system for the investigation of nuclear inactivation by environmental agents. Parag and Giami (1975) have already shown that following ultraviolet irradiation of dikaryotic mycelia of *Schizophyllum commune*, the frequency of monokaryons among survivors is very much lower than would be expected from nuclear inactivation. Their results indicate the resolving power of this system.

FIG. 1.—Frequencies of monokaryotic mycelial fragments among survivors when nuclei are inactivated independently of each other. The continuous line represents the theoretical curve for unicellular fragments ($n = 1$): $P_s = 1 - p^2$; $P_m = \frac{2p(1-p)}{1-p^2}$. Points ● represent the heterogeneous population with $\bar{n} = 3$; points ▲ represent the heterogeneous population with $\bar{n} = 10$. P_s and P_m are calculated from formulas (4) and (3) respectively. In these heterogeneous populations, the different fragment sizes are assumed to be randomly distributed around the mean $\bar{n}$. The respective probabilities of nuclear inactivations p_1, p_3, p_{10} corresponding to the survival frequencies of the three populations are given below the abscissae. Points X represent a mixture of $n = 1$ and $n = 10$; extreme deviations for each of five different values of p ($p = 0.8; 0.9; 0.95; 0.97; 0.99$).

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