Itoh, T. and K. Morishita. Cytoplasmic contribution to protoperithecium formation in N. crassa.

Jinkr (1957 Proc. Roy. Soc. London Se,. B 146:527) suggested that, in Aspergillus glaucus, although phenotypic variations (wiring during unselected asexual propagation of the selected liner and established by selection) ore purely cytoplasmic in origin, variation of fertility can depend on physiological conditions. Fitzgerald (1963 Heredity 18:47), moreover, states that inability to form protoperithecia in a sterile strain of Neurospora, which is controlled by two genes at least, and the variation of the phenotypic expression ore not due to gene differences but appear to be determined by different equilibrium states, some of which are self-perpetuating under certain conditions.

We frequently find that the formation of protoperethecia decreases spontaneously, so that fruiting bodies become scarce, in cultures of N. crassa. Results from successive subcultures of strains varying in the frequency of formation of protoperithecia provide apparent evidence of a gradual decrease (Table 1). A culture that forms few perithecia reaches the zero level more rapidly in the course of serial subcultures than does a culture that forms protoperithecia abundantly. Moreover, subcultivation by the successive isolation of single conidio also results in a gradual decrease of protoperithecium formotion (Table 2). Accordingly, it is probably preferable to soy that the change in subsequent cultures stems either from effects of environmental factors on gene action or from abnormalities of the cellular systems of conidia and mycelia as stated by Jinkr ond Fitzgerald.

To determine whether the factor for phenotypic expression is genic or cytoplasmic, reciprocal crossing would be the best type of experiment. As shown in Table 3, the distribution of the frequency among ascospore segregants was obviously different between paired reciprocal crosses involving non-protoperithecial strains 8A, 13A and protoperithecial strains 28a, 120, 220 and 32a. In other words, there were many more progeny protoperithecia in the first cross than in the second, and more progeny not forming protoperithecia in the second than in the first (Table3). Therefore, it is possible to soy that the cytoplasm of the maternal strain, which will be used for forming ascospores, contoinr many more factors which influence protoperithecium formation than do the nuclei of those strains which ore being crossed with them.

Table 1. Protoperithecium formation in successive transfers of moss spores of several strains.

Strain	Transfers								
	1	2	3	4	5	6	7	8	9
730	*100	IO	IO	0	0	0	0	0	0
STA4	100	10	IO	0	0	0	0	0	0
40a	200	200	100	10	0	0	0	0	0
7a	500	200	100	IO	0	0	0	0	0
8a	600	-	-	200	100	IO	0	0	0
17a	900	-	-	-	300	200	100	IO	0

*Number of protoperithecia produced around a small filter paper circle (1 cm. d.) put on agar crossing medium and counted 5-6 days after inoculation.

Table 2. Protoperithecium formotion through three successive transfers of single spores of strain @a.

Transfer	No. of cultures producing perithecia at given level					
	0	IO	100	200	300	400
I*(600)	IO	16	16	5	1	0
II (300)	25	6	3	2	0	0
III (200)	32	2	1	0	0	0

'Level of perithecium production by culture from which spore inoculum was token.

Table 3. Segregation of level of protoperithecium formation from reciprocal crosses of strains derived from 74A and 73a.

Parental Strains		Level of protoperithecium formation					
Maternal	Paternal	0	10	100	200	300	400
28a (10)	x 8A (0)	70	26	7	4	1	0
12a(10)	x 13A (0)	62	13	8	7	0	0
22a(100)	x 8A (0)	59	26	14	9	1	0
32a(100)	x 13A (0)	28	36	15	14	8	4
8A (0)	x 28a (10)	105	4	1	0	0	0
13A (0)	x 12a (10)	91	3	1	1	0	0
8A (0)	x 22a (loo)	107	4	3	1	0	0
13A (0)	x 32a (100)	101	5	1	0	0	0

Table 4. Variation in level of protoperithecium formation of monospore and hyphal tip isolates from strains STA4 and 12a.

Strain	Number of isolates producing protoperithecia at level						
	0	10	100	200	300	400	500
0. Mono-spore isolation							
STA4 (100)	31	13	0	0	0	0	0
12a (600)	10	16	16	1	1	0	0
b. Hyphol-tip isolation							
STA4 (100)	18	16	3	6	1	0	0
12a (600)		32	21	7	4	1	1

Even though the influential factors in protoperithecium formation ore first and foremost cytoplasmic rather than genic, the problem was left unsolved as to whether the variation seen in subsequent propagation results from external effects of environmental factors or from abnormalities of the cellular system. In order to investigate this point, monospore and hyphal-tip isolation tests were carried out (Table 4). The protoperithecial formation of an isolated conidium varied, never equally or surpassing the number formed by the parental clone. In monospore isolation, the maximum level of formation was from 10 to 3 x 10^2, and in hyphal-tip isolation it was 3-5 x 10^2. The variation shown in monospore and hyphal-tip isolation tests seems to depend upon an irregular distribution of the cytoplasmic factors responsible for protoperithecium formation. Therefore, it seems that the variation seen in subsequent propagation (Table 2) results from irregular distribution of cytoplasmic factors and a change of cellular physiological state depending upon the distribution of the factors during subculture, rather than resulting from external effects of environmental factors. In conclusion, the observations reported here would therefore seem to support the view that the factors for protoperithecium formation are cytoplormic, that they are inherited only when they are carried by the protoperithecial parent, and are distributed irregularly into conidia produced from mycelia. The possibility that something is being transmitted through the cytoplasm is immediately suggested. However, conclusions regarding the mechanism of its variation must await further investigation. ▪ ▪ ▪ Biology Laboratory, Obihiro Zootechnical University, Cbihim, Hokkoido, Japan.