The inheritance of the killer character in yeast

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1. INTRODUCTION

The killer reaction in *Saccharomyces cerevisiae* was discovered by Bevan & Makower (1963), who described how strains of this yeast could be classified into one of three phenotypes with respect to the killer character, viz killer, sensitive or neutral. When killer and sensitive cells are grown together in the same culture medium, a high proportion of the latter are killed. Neutral cells are not killed by killer cells, nor do they kill sensitive cells.

Cell contact is not required for the killing reaction to occur: medium in which killer cells have previously been grown and removed by filtration retains its ability to kill sensitive cells. The agent released by killer cells which brings about the death of sensitive cells has been called the 'killer factor'. Woods & Bevan (1968) showed that the killer factor is an unstable macromolecular protein, and defined the conditions necessary for the production of stable high titre killer solutions.

The present work describes a genetic analysis of our laboratory stocks of killer, neutral and sensitive yeast strains. These studies show that the killer character is under the control of two types of cytoplasmic genetic determinant (k) and (n), both of which require the presence of the dominant nuclear allele *M* for their maintenance in the cell.

2. MATERIALS AND METHODS

(i) *Strains*. The genotypes and designations of the strains used in this analysis are listed in Table 1. All originated from the wild-type haploid strains WT_4 and WT_5 of the Oxford stock collection (Woods & Bevan, 1966).

(ii) *Media*. Agar complete medium (ACM) (Cox & Bevan, 1962) was used for genetic crosses and ascus dissection. The pH of this medium is 5·8. Incubation temperature was 28 °C.

Acetate medium was used to induce diploid cells to sporulate. Incubation temperature was 24 °C.

Buffered methylene blue agar complete medium (MBM) was used for phenotype testing. Since the killer factor is stable only between pH 4·6 and 4·8 (Woods & Bevan, 1968), this medium was buffered at pH 4·8 with a phosphate-citrate

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buffer. Methylene blue, a specific stain for dead yeast cells (Lindegren, 1949), was incorporated into the phenotype testing medium at a final concentration of 0·003 %. Incubation temperature was 24 °C.

Table 1. *Strains of* Saccharomyces cerevisiae *used in the present study*

Strains	Mating type	Auxotrophic requirements	Phenotype
K1, K2, K3	α	WT	
K4, K5, K6	a	WT	
K7	a	arg–9	Killer
K8	α	ad–1.3	
K9	α	ad–1.4	
N1	a	WT	
N2	α	WT	Neutral
S1, S2, S3, S4, S6	α	WT	
S5, S7	a	WT	Sensitive

ad and *arg* indicate requirements for adenine and arginine respectively. *a/α*: the two alleles of the mating type locus. WT Wild-type strains.

(iii) *Micromanipulation.* A Singer micromanipulator (Barer & Saunders-Singer, 1948) was used for single cell mating and ascus dissection. Microloops with a straight shaft and loops of approximately $10\,\mu$ diameter were made on a de Fonbrunne microforge (de Fonbrunne, 1949). The open plate method of micromanipulation (Cox & Bevan, 1961) was used.

(iv) *Genetic crosses and ascus dissection.* All genetic crosses were performed by single cell mating. The first diploid cell produced by the mating figure was isolated and incubated on ACM for 3 days. Part of the resulting clone was tested for phenotype, and the remainder was incubated on sporulation medium for 3 days.

Prior to dissection, asci were suspended in mushroom enzyme (Bevan & Costello, 1964). After 1–2 h incubation at 28 °C the ascus walls were dissolved, leaving the spores intact and adhering together as tetrads. Isolated spores were incubated on ACM for 3 days before being tested for phenotype.

(v) *Phenotype tests* (Plate 1). Three-day-old clones to be tested for phenotype were either replica plated or transferred with a loop on to each of two MBM plates which had previously been spread respectively with a thin background lawn of diploid killer and sensitive cells of stock strains. Following 2 days' incubation, colonies of the sensitive phenotype were defined on the killer background lawn, the sensitive cells having been killed by the background and hence stained by the methylene blue. Colonies of the killer phenotype were detected on the sensitive background lawn, being surrounded by a zone of clear agar due to the death of the sensitive cells within the zone of diffusion of the killer factor. Colonies of the neutral phenotype gave no reaction on either background lawn.
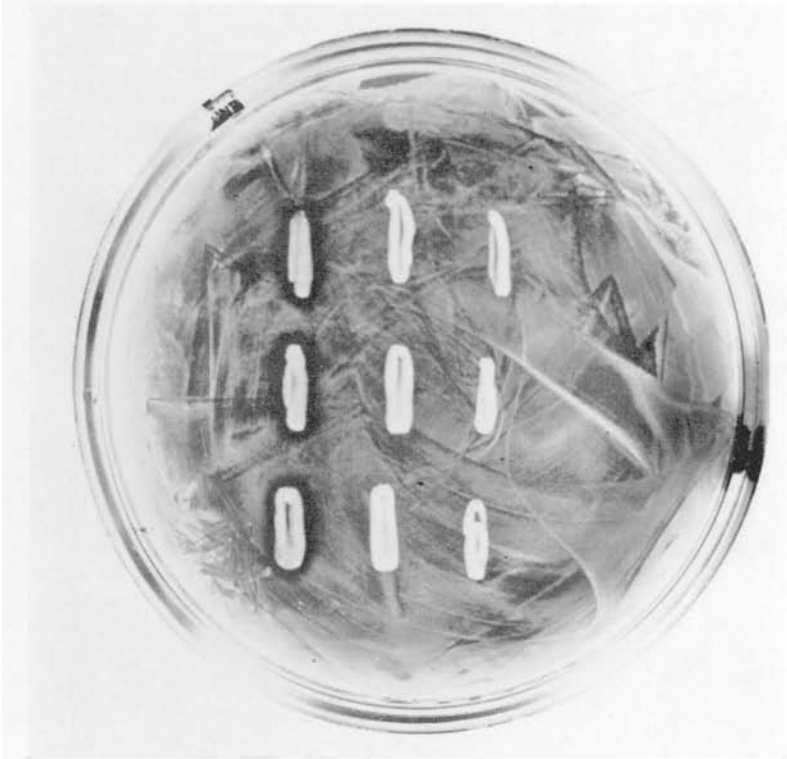
Killer background
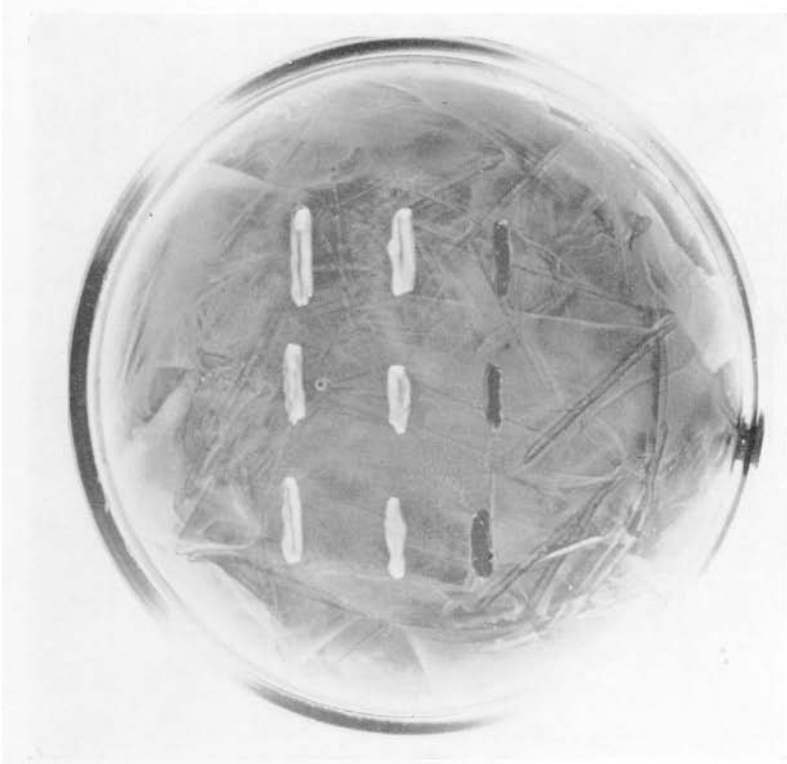
Sensitive background

Fig. 2. Similar samples inoculated on to MBM medium previously spread with a lawn of killer cells.

Fig. 1. Samples of killer (K), neutral (N) and sensitive (S) cells inoculated on to the MBM medium previously spread with a lawn of sensitive cells.

3. RESULTS

(i) *Killer by sensitive crosses*

Single cell matings between killer and sensitive cells are possible because the sensitive cell is not killed within the time required for mating (about 3 h) at the pH (5·8) of the ACM on which the cells are mated. Table 2 summarizes the results of the first generation crosses. In each cross the diploid possessed the killer phenotype. All crosses involving strains S6 and S7 yielded only killer spores, suggesting that the killer phenotype is inherited cytoplasmically. However, strains S1–S4 yielded predominantly 2:2 tetrad ratios, indicating that the killer character is also under the control of a nuclear gene. It is not clear why such a high percentage of abnormal tetrads were obtained in these crosses (6·9 %) compared with the 'normal' percentage (approximately 2 %) generally obtained from yeast crosses. However, it should be borne in mind that the conclusions derived from these results have been confirmed by further crosses described later.

Table 2. *Tetrad analyses of killer diploids resulting from sensitive*
by killer crosses

Parental strains crossed	Total no. of tetrads analysed	No. of each type of tetrad obtained					Genotypes of parental strains crossed
		4K:0S	3K:1S	2K:2S	1K:3S	0K:4S	
S1 × K4, K5, K7	83	0	3	77	2	1	
S2 × K4, K5, K6, K7	87	0	2	84	1	0	
S3 × K4, K5, K6, K7	62	1	3	56	2	0	$m(o) \times M(k)$
S4 × K4, K5, K7	42	0	2	38	2	0	
S6 × K4, K5, K6	52	52	0	0	0	0	
S7 × K1, K2, K3	50	50	0	0	0	0	$M(o) \times M(k)$

K: killer phenotype. S: sensitive phenotype. M/m: gene determining the presence (k)/absence(o) of killer cytoplasmic determinants.

On the basis of these preliminary results the following working hypothesis was adopted. The killer phenotype is determined by the presence of cytoplasmic genetic determinants (k), which are maintained or expressed only in the presence of the dominant nuclear allele M. Absence of the (k) determinants, (o), confers the sensitive phenotype, regardless of the nuclear genotype. Hence the genotype of killer strains is $M(k)$, of sensitive strains S6 and S7 $M(o)$, and of sensitive strains S1–S4 $m(o)$.

Second-generation crosses were undertaken to test this hypothesis. In this study, second-generation crosses involved crossing each of the four spore cultures of a tetrad derived from a first-generation cross with standard haploid strains. The results of the second-generation crosses are summarized in Table 3. All $M(k) \times M(k)$ and $M(k) \times M(o)$ crosses yielded only the expected 4K:0S spore ratios. With the exception of one aberrant tetrad, all $M(k) \times m(o)$ crosses gave only the expected 2K:2S spore ratios.

Table 3 also shows that sensitive by sensitive crosses of the types $M(o) \times M(o)$

Table 3. *The results of second-generation killer by sensitive crosses*

Tetrad from cross	Phenotype of spore cultures	Killer M(k)		Sensitive M(o)		Sensitive m(o)		Confirmed genotype of spore culture
		Diploid phenotype	Spore ratios obtained*	Diploid phenotype	Spore ratios obtained*	Diploid phenotype	Spore ratios obtained*	
m(o) × M(k)	Sensitive	Killer	2K:0S (10)	Sensitive	0K:4S (6)	Sensitive	0K:4S (8)	m(o)
	Sensitive	Killer	2K:2S (7)	Sensitive	0K:4S (10)	Sensitive	0K:4S (10)	m(o)
	Killer	Killer	4K:0S (10)	Killer	4K:0S (12)	Killer	2K:2S (8)	M(k)
	Killer	Killer	4K:0S (9)	Killer	4K:0S (5)	Killer	2K:2S (11)	M(k)
M(o) × M(k)	Killer	Killer	4K:0S (5)	Killer	4K:0S (13)	Killer	2K:2S (10)	M(k)
	Killer	Killer	4K:0S (11)	Killer	4K:0S (13)	Killer	2K:2S (12)†	M(k)
	Killer	Killer	4K:0S (12)	Killer	4K:0S (14)	Killer	2K:2S (10)	M(k)
	Killer	Killer	4K:0S (8)	Killer	4K:0S (11)	Killer	2K:2S (11)	M(k)

* The number of tetrads analysed is given in parentheses.

† One aberrant tetrad was isolated.

and $M(o) \times m(o)$ yield sensitive diploids and spores only. Failure to obtain killer diploids from crosses of the type $M(o)$ sensitive by a $m(o)$ sensitive derived from a killer diploid $Mm(k)$ suggests that (k) determinants are not present in $m(o)$ sensitive strains, and rules out the alternative possibility that (k) determinants are maintained but not expressed in the presence of the nuclear allele m.

Confirmatory evidence that (k) determinants are not maintained in the presence of m was obtained by sporulating a diploid culture of genotype $Mm(k)$ (resulting from a cross of the type $M(k) \times m(o)$), and analysing the cells derived from each

Table 4. *The results of crossing spore-derived cells of two tetrads from a diploid* Mm(k) *with* M(o) *sensitive cells*

Tetrad	2-day-old colony		No. of cells in micro- colony	No. of cells crossed with $M(o)$	Diploid phenotypes	Genotypes of spore derived cells crossed
	Phenotype	Genotype				
1	Sensitive	$m(o)$	19	4	2 killer	$m(k)$
					2 sensitive	$m(o)$
1	Sensitive	$m(o)$	18	4	4 killer	$m(k)$
	Killer	$M(k)$	22	8	8 killer	$M(k)$
	Killer	$M(k)$	23	10	10 killer	$M(k)$
2	Sensitive	$m(o)$	38	7	5 killer	$m(k)$
					2 sensitive	$m(o)$
2	Sensitive	$m(o)$	55	15	9 killer	$m(k)$
					6 sensitive	$m(o)$
	Killer	$M(k)$	23	8	8 killer	$M(k)$
	Killer	$M(k)$	30	8	8 killer	$M(k)$

of the 4 spores of two tetrads. After allowing each spore to divide into a micro-colony of between 18 and 55 cells, a number of individual cells from each colony were isolated and left to cross with both a and $\alpha M(o)$ sensitive cells. (The mating types and phenotypes of the microcolonies were not known at this stage.) The remaining cells of each microcolony were left to form a 2-day-old colony. The haploid spore cultures and the diploid cultures which resulted from the crosses were then tested for phenotype.

The results of these studies are summarized in Table 4. the original presence of (k) determinants in each spore which eventually grew to give a sensitive clone after 2 days is shown by the isolation of killer diploids when cells of the microcolony were crossed with $M(o)$ cells of opposite mating type. In effect crosses of the type $m(k) \times M(o)$ resulted in the formation of killer diploids of $Mm(k)$ genotype, which on subsequent tetrad analyses gave 2K:2S spore ratios as expected. That the (k) determinants are not maintained indefinitely in the presence of the allele m is shown by the fact that a number of diploids were also isolated which had the sensitive phenotype, and hence were the result of a cross of the type $m(o) \times M(o)$. Thus, although (k) determinants may persist for a limited time in the presence of the allele m, they begin to be lost from the cells after 4–5 cell generations.

(ii) *Neutral by sensitive crosses*

Table 5 shows the results of crossing sensitive strains S1–S4, S6 and S7 with the two stock neutral strains, N_1 and N_2. All diploids possessed the neutral phenotype. Crosses involving strains S6 and S7 yielded neutral spores only, suggesting that the neutral phenotype is inherited cytoplasmically. Strains S1–S4, however, showed single gene inheritance for the neutral phenotype. This striking parallel with the results of the killer by sensitive crosses leads us to propose that a second type of cytoplasmic genetic determinant, (n), determines the neutral phenotype, and that the maintenance of (n) is under gene control. Moreover, the genotypes of strains S1–S4 are such that they are unable to maintain either (k) or (n) determinants when introduced by crossing, whereas strains S6 and S7 are able to maintain both types of determinant. Hence no evidence has been obtained to suggest that (k) and (n) are under the control of separate nuclear genes, and the genotype of neutral strains will therefore be represented as M(n).

Table 5. *Tetrad analyses of neutral diploids resulting from sensitive by neutral crosses*

Parental strains crossed	Total no. of tetrads analysed	No. of each type of tetrad obtained					Genotypes of parental strains crossed
		4N:0S	3N:1S	2N:2S	1N:3S	0N:4S	
S1 × N1	12	0	0	12	0	0	
S2 × N1	8	0	1	7	0	0	m(o) × M(n)
S3 × N1	18	0	1	17	0	0	
S4 × N1	12	0	0	12	0	0	
S6 × N1	18	18	0	0	0	0	M(o) × M(n)
S7 × N2	9	9	0	0	0	0	

S: sensitive phenotype; N: neutral phenotype; M/m: gene determining the presence (n)/absence (o) of neutral cytoplasmic determinants.

The second-generation crosses performed are summarized in Table 6. The results exactly parallel to those of the second-generation killer by sensitive crosses summarized in Table 3. Again, crosses of the type M(o) sensitive by an m(o) sensitive derived from an Mm(n) diploid resulted in sensitive diploids, thus suggesting that (n) determinants are not maintained in the presence of the allele m.

To show that the sensitive spore cultures resulting from a neutral diploid of genotype Mm(n) originally receive (n) determinants, (genotype m(n)), and that these are not maintained in the presence of the recessive allele, m, crosses of the type m(n) × M(o) were carried out as described in section (i). The results of these are summarized in Table 7, and show that the loss of (n) determinants has begun by the 5th cell generation.

(iii) *Killer by killer crosses*

Killer strains K1–K7 were crossed together in various combinations. From a total of nine crosses, all diploids were of killer phenotype, and of the 144 tetrads

Table 6. *The results of second generation neutral by sensitive crosses*

Tetrad from cross	Phenotype of spore cultures	Spore cultures crossed with							Confirmed genotype of spore culture
		Neutral *M*(n)		Sensitive *M*(o)		Sensitive *m*(o)			
		Diploid phenotype	Spore ratios obtained*	Diploid phenotype	Spore ratios obtained*	Diploid phenotype	Spore ratios obtained*		
m(o) × *M*(n)	Sensitive	Neutral	2N:2S (13)	Sensitive	0N:4S (11)	Sensitive	0N:4S (12)		*m*(o)
	Sensitive	Neutral	2N:2S (13)	Sensitive	0N:4S (11)	Sensitive	0N:4S (12)		*m*(o)
	Neutral	Neutral	4N:0S (10)	Neutral	4N:0S (10)	Neutral	2N:2S (12)		*M*(n)
	Neutral	Neutral	4N:0S (7)	Neutral	4N:0S (4)	Neutral	2N:2S (8)		*M*(n)
M(o) × *M*(n)	Neutral	Neutral	4N:0S (11)	Neutral	4N:0S (10)	Neutral	2N:2S (10)		*M*(n)
	Neutral	Neutral	4N:0S (8)	Neutral	4N:0S (11)	Neutral	2N:2S (6)		*M*(n)
	Neutral	Neutral	4N:0S (12)	Neutral	4N:0S (10)	Neutral	2N:2S (9)		*M*(n)
	Neutral	Neutral	4N:0S (10)	Neutral	4N:0S (12)	Neutral	2N:2S (9)		*M*(n)

* The number of tetrads analysed is given in parentheses.

analysed, all yielded 4K:0S tetrad ratios. Second-generation crosses of a 4K:0S tetrad with both M(o) and m(o) sensitive strains gave only the expected tetrad ratios of 4K:0S and 2K:2S respectively. Between six and 12 tetrads from each of the four diploids resulting from the second-generation crosses were analysed.

Table 7. *The results of crossing spore-derived cells of a tetrad from a diploid* Mm(n) *with* M(o) *sensitive cells*

2-day-old colony		No. of cells in micro-colony	No. of cells crossed with M(o)	Diploid phenotype	Genotypes of spore derived cells crossed
Phenotype	Genotype				
Sensitive	m(o)	34	10	4 neutral 6 sensitive	m(n) m(o)
Sensitive	m(o)	33	13	7 neutral 6 sensitive	m(n) m(o)
Neutral	M(n)	36	6	6 neutral	M(n)
Neutral	M(n)	59	19	19 neutral	M(n)

(iv) *Neutral by neutral crosses*

The two neutral strains N1 and N2 were crossed. The resulting diploid had the neutral phenotype, and of the 11 tetrads analysed, all gave tetrad ratios of 4N:0S. Second-generation crosses of one 4N:0S tetrad with both M(o) and m(o) sensitive strains yielded only the expected tetrad ratios of 4N:0S and 2N:2S respectively. Between six and 12 tetrads from each diploid were analysed following the second-generation crosses.

(v) *Sensitive by sensitive crosses*

The three genotypically different types of sensitive by sensitive cross were performed, viz M(o) × M(o), M(o) × m(o), and m(o) × m(o). Each yielded a sensitive diploid; 10, 29 and 13 tetrads respectively were analysed and all gave only sensitive spores. The result of the second-generation crosses undertaken are summarized in Table 8. They show that normal segregation occurred with respect to the M/m gene. The sensitive spore cultures analysed were either able to maintain both (k) and (n) when introduced by crossing (i.e. they were genotype M(o)), or neither type of cytoplasmic determinant (i.e. they were genotype m(o)). Hence no evidence was obtained to suggest that (k) and (n) are under the control of separate genes.

(vi) *Killer by neutral crosses*

According to the proposed genetic model, killer by neutral crosses are expected to result in the formation of diploid cells containing both types of cytoplasmic determinants, and which are homozygous for the nuclear allele M, i.e. are genotype MM(k)(n). The results of the first-generation crosses performed are shown in Table 9. All diploids had the killer phenotype, as did the majority of spore cultures, but a small number of neutral spores were also obtained. Normal segregation

occurred with respect to the accompanying adenine and arginine nuclear markers. These results are consistent with the hypothesis that both (k) and (n) determinants are maintained in the cells of these diploid cultures, but that the presence of the

Table 8. *The results of second generation sensitive by sensitive crosses*

		Spore cultures crossed with				
		Killer M(k)		Neutral M(n)		Confirmed genotype
Tetrad from cross	Phenotype of spore cultures	Diploid phenotype	Spore ratios obtained*	Diploid phenotype	Spore ratios obtained*	of spore culture
m(o) × m(o)	Sensitive	Killer	2K:2S (12)	Neutral	2N:2S (13)	
	Sensitive	Killer	2K:2S (12)	Neutral	2N:2S (14)	m(o)
	Sensitive	Killer	2K:2S (12)	Neutral	2N:2S (9)	
	Sensitive	Killer	2K:2S (12)	Neutral	2N:2S (9)	
M(o) × M(o)	Sensitive	Killer	4K:0S (6)	Neutral	4N:0S (7)	
	Sensitive	Killer	4K:0S (7)	Neutral	4N:0S (9)	M(o)
	Sensitive	Killer	4K:0S (7)	Neutral	4N:0S (7)	
	Sensitive	Killer	4K:0S (10)	Neutral	4N:0S (9)	
M(o) × m(o)	Sensitive	Killer	4K:0S (7)	Neutral	4N:0S (10)	M(o)
	Sensitive	Killer	4K:0S (9)	Neutral	4N:0S (12)	M(o)
	Sensitive	Killer	2K:2S (12)	Neutral	2N:2S (5)	m(o)
	Sensitive	Killer	2K:2S (12)	Neutral	2N:2S (10)	m(o)
M(o) × m(o)	Sensitive	Killer	4K:0S (9)	Neutral	4N:0S (6)	M(o)
	Sensitive	Killer	4K:0S (11)	Neutral	4N:0S (12)	M(o)
	Sensitive	Killer	2K:2S (11)	Neutral	2N:2S (7)†	m(o)
	Sensitive	Killer	2K:2S (6)	Neutral	2N:2S (11)†	m(o)

* The number of tetrads analysed is given in parentheses.
† One aberrant tetrad was isolated.

Table 9. *Tetrad analyses of killer diploids resulting from killer by neutral crosses*

Parental strains crossed	Total no. of tetrads analysed	No. of each type of tetrad obtained				
		4K:0N	3K:1N	2K:2N	1K:3N	0K:4N
K1 × N1	27	21	5	1	0	0
K2 × N1	22	22	0	0	0	0
K3 × N1	37	18	8	3	7	1
K4 × N2	17	12	5	0	0	0
K5 × N2	17	17	0	0	0	0
K6 × N2	16	16	0	0	0	0
K7 × N2	5	5	0	0	0	0
K8 × N1	24	24	0	0	0	0
K9 × N1	27	9	8	4	4	2

(n) determinants is masked by the over-all phenotype of the cultures. Somatic segregation of (k) and (n) may be expected to occur during vegetative growth of these diploid cultures, resulting in a continuous range in the relative proportions of (k) and (n) per cell. Thus 4K:0N tetrad ratios would be expected from diploid cells with a high proportion of (k) determinants; 0K:4N ratios from diploid cells

containing no, or perhaps very few, (k) determinants; and intermediate spore ratios from cells containing intermediate proportions of (k) and (n). Three types of spore are thus to be expected from these crosses: killer spores $M(k)$, killer spores $M(k)(n)$ and neutral spores $M(n)$.

Supporting evidence for such maintenance of (k) and (n) together in the cells, and for their somatic segregation during the vegetative growth of diploid cells of genotype $MM(k)(n)$, was obtained from further crosses. Intra-ascus and back-crosses of each of the spores of nine tetrads were performed. The data obtained from one 4K:0N and one 1K:3N are presented in Tables 10 and 11 respectively and serve to illustrate the significant points arising from these studies.

Table 10. *The results of intra-ascus and back crosses of a 4K:0N tetrad obtained from a first generation killer by neutral cross*

Strains crossed	Diploid phenotype	Total no. of tetrads analysed	No. of each type of tetrad obtained				
			4K:0N	3K:1N	2K:2N	1K:3N	0K:4N
A killer spore × B killer spore	Killer	9	1	2	1	4	1
B killer spore × C killer spore	Killer	14	9	2	2	1	0
A killer spore × D killer spore	Killer	8	7	1	0	0	0
C killer spore × D killer spore	Killer	8	2	1	2	1	2
Neutral parent × A killer spore	Killer	6	6	0	0	0	0
Neutral parent × C killer spore	Killer	9	5	2	1	1	0
Killer parent × B killer spore	Killer	11	11	0	0	0	0
Killer parent × D killer spore	Killer	9	9	0	0	0	0

Table 10 shows that all four killer by killer intra-ascus crosses gave killer diploids, but that a high number of neutral spores were recovered in addition to killer spores. These results resemble first-generation killer by neutral crosses, which gave killer diploids of genotype $MM(k)(n)$ which when spored yield a proportion of neutral spores. They differ from the results of the first generation killer by killer crosses detailed in section (iii) which yielded killer diploids $MM(k)$ which when spored gave killer spores only. Thus these findings suggest that killer spores of genotype $M(k)(n)$ are produced from first-generation killer by neutral crosses, and that when intra-ascus crosses are carried out $M(k)(n)$ killer × $M(k)(n)$ killer crosses produce diploids of genotype $MM(k)(n)$ which on sporulation yield a proportion of neutral spores.

Further evidence for the production of $M(k)(n)$ killer spores is seen in Table 11, which shows the results of intra-ascus and back crosses of each of the spore cultures from a 1K:3N tetrad obtained from a first-generation killer $M(k)$ × neutral $M(n)$ cross. When killer spore culture B was backcrossed to the neutral parent strain, the first diploid cell isolated gave rise to a neutral culture and neutral spores only, thus suggesting that the cell taken for crossing from the killer spore culture was a neutral, $M(n)$. On repeating this cross a killer diploid was obtained, indicating that the cell taken for crossing in this instance contained a number of (k) determinants. Similarly, the intra-ascus cross C neutral × B killer yielded a neutral diploid, but this cross was not repeated.

Direct evidence for the somatic segregation of (k) and (n) from haploid and diploid vegetative cultures derived from killer by neutral crosses will be presented in a later publication.

Table 11. *The results of intra-ascus and back crosses of a* $1K:3N$ *tetrad obtained from a first generation killer by neutral cross*

Strains crossed	Diploid phenotype	Total no. of tetrads analysed	No. of each type of tetrad obtained				
			4K:0N	3K:1N	2K:2N	1K:3N	0K:4N
A neutral spore × B killer spore	Killer	10	1	3	0	1	5
A neutral spore × D neutral spore	Neutral	8	0	0	0	0	8
B killer spore × C neutral spore	Neutral	17	0	0	0	0	17
C neutral spore × D neutral spore	Neutral	11	0	0	0	0	11
Neutral parent × B killer spore	Neutral	10	0	0	0	0	10
Neutral parent × B killer spore	Killer	7	6	0	1	0	0
Neutral parent × D neutral spore	Neutral	10	0	0	0	0	10
Killer parent × A neutral spore	Killer	10	5	3	2	0	0
Killer parent × C neutral spore	Killer	10	10	0	0	0	0

4. DISCUSSION

The results of the crosses described here support the hypothesis that the killer character in yeast is under the control of a nuclear gene M/m and two types of cytoplasmic genetic determinants, (k) and (n). The presence of (k) determinants confers the killer phenotype, and the presence of (n) determinants the neutral phenotype. The results of the crosses described in section (vi) indicate that (k) and (n) may be present together in the same cell; such a culture has the killer phenotype. Absence of both types of determinant, (o), confers the sensitive phenotype.

Both the (k) and (n) determinants are under gene control. No evidence has been obtained from the present study to suggest that they are controlled by separate nuclear genes, since the genotypes of all the sensitive strains analysed are such that they are able to maintain either both, or neither, type of determinant. Recent gene-mapping studies (K. Theivendirarajah & E. A. Bevan, in preparation) have confirmed that both (k) and (n) are controlled by the same gene M/m.

The present studies have shown that the allele M is required for the maintenance of (k) and (n) in the cytoplasm, as opposed to the alternative hypothesis that the cytoplasmic determinants are present but not expressed in the presence of the allele m. This conclusion is based on the finding that when the nuclear genotype is changed from M to m, loss of the cytoplasmic determinants was seen to occur at about the fourth- or fifth-cell generation, by the failure of the cells to 'complement' with M(o) sensitive cells to form a killer diploid Mm(k). These results also confirm the presence of cytoplasmic determinants in killer and neutral cells and preclude the alternative 'polygene' hypothesis which was considered by Ephrussi, Hottinguer & Taulitzki (1949) as an alternative interpretation to the results of

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their genetic studies on the 'petite' mutation in yeast. The mutagenic action of acriflavine on killer and neutral cells (J. M. Somers & E. A. Bevan, in preparation) further supports the cytoplasmic theory of inheritance.

The possibility that the killer and neutral phenotypes are controlled by one type of cytoplasmic determinant which is present in different numbers in killer and neutral cells is considered unlikely. Chance variation in the number of determinants per cell would be expected to occur, and would result in the production of killer cells from neutral strains, and vice versa. Of the many hundreds of neutral cells plated out during the course of experimental work, none have been observed to give killer colonies. Instances where neutral colonies have been isolated from killer strains are thought to be due to somatic segregation of (k) and (n) in an M(k)(n) killer culture. Furthermore, the maintenance of a constant number of determinants per cell would have to be under the control of the determinants themselves, since M(o) sensitive strains may express either the killer or the neutral phenotype, according to the determinants which are introduced by genetic crosses. This in itself implies that two different types of determinant are involved.

Nevertheless, if the killer character is under the control of two different types of cytoplasmic determinant, it seems probable that they resemble each other closely; indeed, one may be a mutant form of the other. They have at least two properties in common; both are maintained by the same nuclear gene, and both confer immunity to the killer factor.

The identity of (k) and (n) is at present unknown. Electron microscope studies are in progress to determine whether or not visible particles are present in the cytoplasm of killer and neutral cells. It is, however, considered unlikely that (k) and (n) are associated with the cytoplasmic rho particles determining respiratory competence in yeast, since the loss of (k) and (n) in the presence of the allele m is not accompanied by the loss of rho particles; m(o) sensitive cultures are respiratory sufficient.

<div align="center">SUMMARY</div>

1. First- and second-generation crosses between killer, neutral and sensitive strains of yeast have been carried out in all combinations.

2. The results of this analysis indicated that the killer character is under the control of two types of cytoplasmic genetic determinant. One type, (k), determines killing, and the other, (n), neutrality. The absence, (o), of both types of determinants confers the sensitive phenotype.

3. That both types of cytoplasmic determinant require the same dominant nuclear allele, M, for their maintenance has been indicated in two ways. First, both types are lost when the nuclear genotype is changed from M to m. Secondly, cells of genotype m(k) or m(n), which have been shown to occur among the first formed cells arising from spores of Mm(k) and Mm(n) diploids respectively, are unable to maintain their cytoplasmic determinants. On the other hand, spore cultures of M(k) and M(n) genotype derived from these same diploids continue to maintain the determinants.

4.* Thus genotype of killer cells is $M(k)$, of neutrals $M(n)$, and of sensitivities either $M(o)$ or $m(o)$.

5. Cells maintaining both types of cytoplasmic determinant (i.e. of genotype $M(k)(n)$ or $M-(k)(n)$) have been obtained by appropriate crosses, and shown to be of killer phenotype.

6. Alternative hypotheses to account for the results of this genetic analysis have been discussed.

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* The genetic basis of the killer character given here differs from that originally proposed by Bevan & Makower (1963). This is due to the analysis of a larger number of strains in the present study which has revealed the existence of $m(o)$ sensitive strains not previously detected.

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