Proc. Nat. Acad. Sci. USA
Vol. 72, No. 11, pp. 4546–4549, November 1975
Genetics

Genetic analysis in the dinoflagellate *Crypthecodinium* (*Gyrodinium*) *cohnii*: Evidence for unusual meiosis

(motility mutants/complementation/recombination/tetrad analysis)

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Communicated by Hans Ris, August 29, 1975

ABSTRACT **The atypical structure and behavior of dinoflagellate chromosomes suggests that the genetics of these organisms might show comparable peculiarities. We have begun genetic analysis of the neritic, marine heterotroph *Crypthecodinium* (*Gyrodinium*) *cohnii* by means of motility mutants that show complementation shortly after zygote formation, permitting identification of heterozygotes. Six mutations, conferring four microscopically distinguishable phenotypes, have been isolated and investigated.**

These "genes" were found to complement in double heterozygotes in all pairwise combinations, indicating that the lesions are recessive and non-allelic. Clones of all possible combinations of these factors have been established and each complements only those combinations expected on the assumption that there are six independent recessive mutant "genes." Tetrad analysis following isolation of over 200 complementing zygotes showed: (*1*) regular segregation with recovery of parental phenotypes and genotypes; (*2*) independent assortment, with one possible exception; (*3*) segregations that were always 1:1; that is, in all tetrads showing recombination, only the two reciprocal recombinant genotypes were found; there were no tetratypes. This behavior could result from centromere linkage or the absence of crossing over in an otherwise conventional meiosis, or it could result from an unusual one-division "meiosis." Some evidence is provided that favors the latter hypothesis.

The chromosomes of dinoflagellates differ from those of typical eukaryotes in several ways. They have no histones (1) and their appearance under the electron microscope is unique with thin DNA strands in a complex, coiled condition, which remains unchanged throughout interphase and mitosis (2). Furthermore, during mitosis, the chromosomes attach to the nuclear membrane rather than to the spindle directly, so that no ordinary centromere exists (3). The recent demonstration of sexuality and genetic recombination in the hardy, marine heterotroph *Crypthecodinium cohnii* (4, 5) offers the possibility of studying the behavior of these chromosomes by genetic analysis. Several questions may be answered by this approach, among which are: Is there evidence of a classical meiosis? Does regular Mendelian segregation occur? Is there genetic evidence of polyploidy or polyteny, concerning which there has been controversy (6, 7)?

Our first report (4) included preliminary evidence of an unusual segregation. We have extended the analysis to six motility determinants, and the results to be presented indicate a regular segregation of genetic information in a 1:1 ratio, inconsistent with segregation patterns given by cytoplasmic inheritance or by polyploid meiosis. The segregation of these markers differs, however, from classical meiosis in that it appears to result from a single division of the zygotic cyst. This division may or may not be followed by one or two additional divisions within the cyst in which there is no further exchange of genetic information.

METHODS

Cultures. For the present study we employed a clone isolated from Provasoli's Woods Hole strain d. Cultures were grown in the medium of Gold and Baren (8) with a natural sea water base either in liquid or solidified with 1.5% Difco Bacto-agar. Stocks were maintained in liquid at 20° and experimental material was grown at room temperature or at 27°. At the latter temperature, growth curves of the wild-type strain during log phase yield doubling times of about 8 hr, independent of illumination.

Mutants. Mutagenesis was achieved by exposure to both UV and nitrosoguanidine. For the former, cells growing in liquid medium were exposed to about 2000 ergs (0.2 mJ)/mm^2 every 48 hr for 1–2 weeks, after which they were plated and incubated. Colonies were transferred to liquid and after 2–3 days they were examined with a dissecting microscope for evidence of aberrant motility. Nitrosoguanidine-treated cells (5) were screened in the same way. Several motility mutants of apparent spontaneous origin were also found. The mutants (called flagella mutants, fm, although precise characterization has not yet been undertaken) were numbered in order of their original isolation. The factors, "genes," were arbitrarily assigned letter designations as will be explained in the results. The mutants and their descriptions are given in Table 4. Wild-type *C. cohnii* swarmers swim at a rate of about 250 μm/sec; slow-swimming mutants moved no faster than 50 μm/sec.

While the number of mutants is small, it is clear that some lesions have reappeared independently. On three separate occasions, a clone was found to be doubly mutated. In two of these cases, however (fm 1 and fm 3) there is good evidence, based on repeated complementation testing, that the original isolate was first singly mutated at the '*a*' locus only, with mutation of the '*b*' factor occurring later, after which the double mutant gradually replaced the single one in the population.

Genetic Methods. As previously described (4), *C. cohnii* is homothallic, so that a means of recognizing heterozygous zygotes is desirable. Fortunately, at least with motility mutants, each factor is recessive to the normal allele brought in by the other gamete, so that a double heterozygote is functionally wild type. This complementation follows fusion within 6–24 hr; the rapidly swimming zygotes stand out clearly in the population of slow swimmers and can be isolated by micromanipulation. Homozygous zygotes also occur within the mating mixture and, while similar in appearance to the heterozygous ones, still swim at a mutant rate. Mixtures older than 24 hr were not used as a source of zygotes because the first wild-type recombinants begin to appear by

Table 1. Analysis of backcrosses of fm 1 × fm 2 segregants*

	Parents	Apparent parental ditype: Type I segregation	Type II segregation		Nonparental ditype
		fm 1/fm 2†	fm 1/fm 2†		WT/fm 2†
a	fm 1	— +	— —		+ —
	fm 2	+ —	+ —		+ —
		fm 1/fm 2 *ab*+ ++*c*	fm 1/fm 2 *a*++ +*bc*	fm 1/fm 2 +*b*+ *a*+*c*	WT/fm 2 +++ *abc*
b	fm 1 *ab*+	— +	— —	— —	+ —
	fm 2 ++*c*	+ —	+ —	+ —	+ —

* + and − signify the presence or absence of wild-type (WT) cells after suitable time for mating and complementation.
† Refers to phenotype of the segregants.

this time and soon become large enough to be confused with the zygotes. After isolation from liquid drops on agar, the zygotes encyst and undergo division within a few hours. The division products of zygotic cysts were routinely separated after 24 hr, grown for several generations in liquid, and analyzed.

Phenotypes were determined by light microscopy. Genotypes were established by testing for complementation. This test was accomplished by mixing 0.1 ml containing approximately 10^4 cells of each of two mutant clones. After 24 hr, the mixtures were examined under a dissecting microscope for the presence of fast-swimming, wild-type cells.

RESULTS

The word *tetrad* will be used to denote the division products of a zygotic cyst, which, like a vegetative one (9), usually contains two, four, or eight cells. Feulgen-stained preparations show no obvious differences between these cysts and the vegetative ones. When isolating tetrads, we occasionally found sixteen cells which undoubtedly arose from one or more mitoses following release of progeny from the cyst. This may also be true of some of the eight-celled tetrads, but in three cases where zygotes were observed at hourly intervals, the eight cells were produced directly without intervening two- or four-cell stages. While nuclei could not be observed in these living cells, Feulgen-stained preparations (4) have indicated that such cells probably proceed through two-, four-, and eight-nucleate stages before emerging as eight cells.

The genetic findings to be presented were obtained from crosses among five mutant strains, fm 1, 2, 13, 17, and 19, and their progeny. When fm 1 and fm 2 were crossed, two kinds of segregations were observed: one consisted of parental phenotypes in a 1:1 ratio (parental ditype) and the other consisted of wild-type and assumed "double mutant" cells (4), an assumption that seemed justified by the finding that these complemented neither parent. When the segregants from the parental ditype tetrads were backcrossed, the results shown in Table 1a indicated that more than one type of segregation had occurred and that the constitution of the parental strains was not so simple as had been supposed. Only Type I was actually a parental ditype. A three-gene model designed to accommodate the different backcross results predicted that the Type II "parental ditypes" were of two kinds (Table 1b). This prediction was fulfilled when the segregants were tested for complementation in all combinations. The results are presented in Table 2.

fm 13, 17 and 19 were crossed with each other and with segregants containing *a*, *b*, and *c* singly or in combination. Complementation results, given in Table 3, indicate that each of the three mutants contained a new lesion; these three were accordingly designated *d*, *e*, and *f*. It can be seen that fm 19 also carried the *a* factor.

Among the mutants and segregants were some whose complementation pattern showed them to be singly marked. These were used as tester strains in establishing the genotypes of other mutants and segregants in terms of a six-factor model. Characterizations of 10 mutants and representative segregants are presented in Table 4. It can be seen from the phenotypes that the factors exhibit epistasis in the following order: $e > d$ and $f > c > a$ and b.

Clones containing various combinations of the six genes were crossed and their tetrads were analyzed using the tester strains. Table 5 shows the frequency with which different tetrad types occurred in two crosses. The results indicate: (*1*) all the genes were recovered; (2) although 2- and 8-celled zygotic cysts were infrequent, they showed no qualitative differences from four-celled cysts; (3) in no case were recombinant and parental genotypes recovered from a single

Table 2. Complementation between segregants from fm 1 × fm 2 crosses

ab+	++*c*	*a*++	+*bc*	+*b*+	*a*+*c*	*abc*	
—	+	—	—	—	—	—	*ab*+
	—	+	—	+	—	—	++*c*
		—	+	+	—	—	*a*++
			—	—	—	—	+*bc*
				—	+	—	+*b*+
					—	—	*a*+*c*
						—	*abc*

Table 3. Complementation between mutant strains and fm 1 × fm 2 segregants

a++	+*b*+	++*c*	*abc*	fm 13	fm 17	fm 19	
+	+	+	+	—	+	+	fm 13
+	+	+	+	+	—	+	fm 17
—	+	+	—	+	+	—	fm 19

Table 4. Characterization of mutants and representative segregants

Strain	Origin	Complementation with tester strains						Phenotype	Assigned genotype
		a	*b*	*c*	*d*	*e*	*f*		
fm 1	Spontaneous	—	—	+	+	+	+	Slow swimming	*ab*
fm 2	UV	+	+	—	+	+	+	Twitching flagella	*c*
fm 3	UV	—	—	+	+	+	+	Same as fm 1	*ab*
fm 12	UV	—	—	+	+	+	+	Same as fm 1	*ab*
fm 13	Spontaneous	+	+	+	—	+	+	Motionless flagella	*d*
fm 14	Spontaneous	+	+	+	—	+	+	Motionless flagella	*d*
fm 15	Spontaneous in an *ac* clone	—	+	—	—	+	+	Motionless flagella	*acd*
fm 17	Nitrosoguanidine	+	+	+	+	—	+	No flagella*	*e*
fm 18	Nitrosoguanidine	+	+	+	+	—	+	No flagella*	*e*
fm 19	Spontaneous in an *a* clone	—	+	+	+	+	—	Same as fm 13	*af*
Segregant	fm 1 × fm 2	—	+	—	+	+	+	Same as fm 2	*ac*
Segregant	*af* × *d*	—	+	+	—	+	—	Same as fm 13	*adf*
Segregant	*abcd* × *e*	—	—	—	—	—	+	Same as fm 17	*abcde*

* As observed using light microscopy.

cyst, i.e., no tetratypes were found. Occasionally, however, reversion to wild type occurred in a clone of a segregant. This produced a "tritype" with two segregant clones of one genotype, a third of complementary genotype, and one containing wild-type cells. These wild-type cells were present, however, as a minority in an otherwise mutant population of cells, which, when isolated and tested, were found to possess the genotype expected of the fourth segregant.

Table 6 has been calculated from the data presented in Table 5 to show segregations of two genes at a time. It can be seen that all the genes, with the possible exception of *b* and *e*, show independent assortment. Recombination between *b* and *e* is rare enough to suggest linkage with a high frequency of crossing over. Clearly more data are needed to prove or disprove this critical point.

Table 5. Tetrad analysis of two crosses

Cross: *ab* × *cd*					Cross: *abcd* × *e*					
Cosegregants	2	4	8†	Total	Cosegregants	2	4	8	16†	Total
a/*bcd*	1	8	2	11	*a*/*bcde*	1	2	1		4
b/*acd*	1	6	1	8	*b*/*acde*		2	1		3
					c/*abde*					0
c/*abd*	1	6	1	8	*d*/*abce*		4			4
					e/*abcd**	1	2	1	1	5
d/*abc*		8	1	9	*ab*/*cde*		2	1		3
ab/*cd**		8	1	9	*ac*/*bde*		1	1		2
					ad/*bce*		1			1
ac/*bd*		4	1	5	*ae*/*bcd*	2	1	1		4
ad/*bc*		5	1	6	*bc*/*ade*		6			6
					bd/*ace*	1	4	1		6
abcd/WT		5	1	6	*be*/*acd*		1			1
					cd/*abe*	1				1
					ce/*abd*		4			4
					de/*abc*	1	1			2
					abcde/WT		1		1	2

* Parental ditype.
† Number of cells from zygotic cyst.

Many crosses in addition to those presented in Tables 5 and 6, involving the *f* factor as well as *a* to *e*, have been analyzed. In over 200 tetrads and over 1000 segregants tested no second division segregation has been observed nor any exception found to the proposed six-gene complementation model.

DISCUSSION

An interpretation of the present findings in classical genetic terms is possibly premature or inappropriate, but will be attempted nevertheless for heuristic reasons. The regularity of segregation of our markers, with no loss of information as in other unusual segregations (10, 11), suggests that division of the zygotic cyst is orderly, comparable to homologue separation in meiosis. A possible physical basis for this regularity may be provided by the nuclear membrane attachment sites of the chromosomes (3, 12). These attachment sites may prove to be involved in pairing, somewhat analogous to the attachment of the synaptonemal complex to the nuclear membrane in conventional meiosis (13, 14), although at present there is no such evidence.

In classical meiosis, the production of only two types of segregants (i.e., segregation at the first division only) could result either from complete absence of crossing over or from close centromere linkage. Precedent exists, however, for another explanation: segregation in *C. cohnii* could occur in one division, prior to chromosome replication. Cleveland (15) has provided cytological evidence for a one-division meiosis in *Oxymonas* and *Saccinobaculus*, multiflagellated parasites of the wood roach *Cryptocercus*, in which both centromere and chromosome duplication were suppressed. Such a division would produce genetic results such as ours. Evidence that reduction in *C. cohnii* does not employ a second meiotic division may be provided by the two-celled zygotic cysts, many of which show recombination (see Table 5). Also the finding of eight-celled cysts (i.e., three divisions) indicates that reduction is not restricted to a conventional two-step meiosis. Strong support for a one-step meiosis awaits more convincing evidence of linkage and crossing over, which would simultaneously eliminate the other two

Table 6. Calculated frequencies from data of Table 5

Cross: *ab* × *cd*			Cross: *abcd* × *e*		
Genes	Parental	Recombinant	Genes	Parental	Recombinant
a and *b*	30 :	32	*a* and *b*	24 :	24
a and *c*	34 :	28	*a* and *c*	23 :	25
a and *d*	34 :	28	*a* and *d*	24 :	24
b and *c*	30 :	32	*a* and *e*	22 :	26
b and *d*	32 :	30	*b* and *c*	28 :	20*
c and *d*	34 :	28	*b* and *d*	27 :	21
			b and *e*	33 :	15†
			c and *d*	20 :	28
			c and *e*	23 :	25
			d and *e*	26 :	22

* $P = 0.2$–0.3 that the ratio equals 1:1.
† $P = 0.01$–0.02.

alternatives and establish the chromosomes as functionally single stranded at the time of crossing over.

Although the life cycle of *C. cohnii* is still imperfectly understood, it seems possible that the occurrence of two-, four-, and eight-celled zygotic cysts could reflect the wide range of DNA content found in different "gametes" and "zygotes" (4). The present genetic evidence indicates that segregation proceeds, nevertheless, as if only two genomes were involved, with none of the irregularities expected of polyploid meiosis. Perhaps *C. cohnii* possesses a mechanism which compensates for genome redundancy. If so, there may be reason to believe that polyteny, which has been proposed (7) in this organism, might be subject to a similar regulation.

We wish to acknowledge the very capable and enthusiastic assistance of two undergraduate research students, Martin Sachs and Fred Leff.

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