GENETIC EVIDENCE OF UNUSUAL MEIOSIS IN THE DINOFLAGELLATE *CRYPTHECODINIUM COHNII*

C. A. BEAM, M. HIMES, J. HIMELFARB, C. LINK AND K. SHAW

Department of Biology, Brooklyn College, Brooklyn, New York 11210

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ABSTRACT

Genetic analysis of the homothallic dinoflagellate, *Crypthecodinium cohnii*, using 16 nonallelic motility mutants, revealed (1) virtual absence of second division segregation and (2) independent assortment of all genes except for: (a) three cases of cross specific, "false" linkage and (b) one possible case of linkage with a high percentage of crossing over. The probability that at least two of the 16 genes studied are on one of the approximately 50 (minimal) chromosomes is extremely high and, since recombination is observed between all pairs of markers, it is highly probable that some results from crossing over. This likelihood plus the observed absence of second division segregation and the significant number of two-celled zygotic cysts support the view that the "meiosis" of *C. cohnii* is a one-division process.

DINOFLAGELLATES differ from other eukaryotes not only in the unusual structure (GRASSÉ *et al.* 1965), behavior (KUBAI and RIS 1969) and biochemistry (RIZZO and NOODEN 1974) of their chromosomes, but also, apparently, in their meiotic behavior. Using motility mutants of the Woods Hole strain d (WH-d) of *Crypthecodinium cohnii*, we have presented evidence of a reduction mechanism (which we called an unusual meiosis) that produces two, four or eight segregants, in which markers are recovered intact, but in which second division segregation is not observed (BEAM and HIMES 1974; HIMES and BEAM 1975). We are aware of good reasons to restrict the term "meiosis" to the well-defined sequence of events established in standard eukaryotic organisms. The word is used in the present context heuristically and in a more general sense, as has been done before (CLEVELAND 1950), to include little understood, perhaps primitive, perhaps basically different processes by which equivalent genomes that have fused, separate without loss of information. Some evidence of linkage and crossing over was also presented, which, if confirmed, would eliminate the two classical interpretations of the absence of second division segregation—centromere linkage and lack of crossing over—and lend support to the previously proposed (HIMES and BEAM 1975) alternative hypothesis, namely that reduction in *C. cohnii* is a one-division process.

In an effort to add to the evidence, we have isolated new strains of *C. cohnii* and produced motility mutants in several of them. Whereas we are mindful of the desirability of conducting genetic studies with stocks as isogenic as possible,

this consideration was in conflict with several others that we regarded as more important at this stage of the investigation. First, we were concerned to discover whether the unorthodox behavior of WH-d was representative of the species, or merely an aberration of the strain. Second, we thought that new isolates might reveal properties that would render them more suitable for genetic studies. Third, having found evidence of sexual incompatibility among established strains, we became interested in diversification within the group and the geographic distribution of gene pools. A preliminary analysis of 13 strains that fall into 8 apparently isolated breeding groups will appear elsewhere (BEAM and HIMES 1977).

Although there is no direct evidence that the motility factors used in this study are located on the cytologically demonstrable chromosomes, the regularity of segregation (HIMES and BEAM 1975) permits interpretation in classical genetic terms. Motility mutants offer two advantages: (1) the phenotypes of individual cells can be distinguished under the microscope, and (2) heterozygous zygotes are easily recognized by virtue of complementation, which is desirable for genetic analysis of a homothallic organism such as *C. cohnii*, especially since there is no way as yet to distinguish between zygotic and mitotic cysts.

The present study represents "tetrad" analysis of 16 nonallelic genes (the original 5, plus 11 new ones). The data presented are from more than 850 segre-

TABLE 1

Strains

Strain	Source	Origin of mutant	Phenotype	Genotype assigned
WHd*	Woods Hole, Mass.	Spontaneous, UV	Slow swimming‡	*a*
WHd*	Woods Hole, Mass.	Spontaneous, UV	Slow swimming‡	*b*
WHd*	Woods Hole, Mass.	UV	Twitching flagella	*c*
WHd*	Woods Hole, Mass.	Spontaneous	Motionless flagella	*d*
WHd*	Woods Hole, Mass.	NTG	No flagella	*e*
WHd*	Woods Hole, Mass.	Spontaneous	Motionless flagella	*f*
WHd*	Woods Hole, Mass.	Spontaneous	Slow swimming‡	*n*
WHd*	Woods Hole, Mass.	X ray	Motionless flagella	*q*
H-1†	Hewlett Point, N.Y.	UV	Slow swimming‡	*g*
H-1†	Hewlett Point, N.Y.	UV	Twitching flagella	*h*
M-4†	Mattapoisett, Mass.	UV	Slow swimming‡	*i*
M-4†	Mattapoisett, Mass.	UV	Gliding motion	*j*
M-4†	Mattapoisett, Mass.	UV	Circular swimming	*k*
M-4†	Mattapoisett, Mass.	UV, NTG	Slow swimming‡	*m*
M-4†	Mattapoisett, Mass.	X-ray	Motionless flagella	*o*
Tr-2†	Trenton, Me.	UV	Slow swimming‡	*l*
PS-1†	Puget Sound, Wash.	Spontaneous	Slow swimming‡	*p*
PS-1†	Puget Sound, Wash.	Spontaneous	Slow swimming‡	*ab*

* Isolated by L. PROVASOLI.
† Original isolate.
‡ These mutants swim at ~50 μm/sec; wild-type strains swim at ~250 μm/sec.

gations from more than 30 different multigene crosses, and, as will be shown, strongly support a one-step meiosis in *C. cohnii* and suggest that its mechanism of recombining linked markers may be unorthodox.

MATERIALS AND METHODS

Cultures: In addition to the widely used WH-d strain of Provasoli (PROVASOLI and GOLD 1962), we have used four new ones that we have isolated—three from the Atlantic seaboard between Maine and New York and one from Puget Sound, Washington—and that are sexually compatible with WH-d. We believe that the Puget Sound strain is the first reported isolation of *C. cohnii* from the Pacific. The axenic strains used and their origins are given in Table 1 together with the mutants derived from them and their characteristics.

The natural sea-water-based medium of GOLD and BAREN (1966) was used exclusively, either liquid or solidified with 1.5% Difco-Bacto Agar. Stock cultures were maintained in liquid medium at room temperature (23°–26°) with uncontrolled illumination and transferred every six weeks. We have found no effect of light on the growth of *C. cohnii* (HIMES and BEAM 1975):

Mutagenesis: Three mutagens were used as previously described (HIMES and BEAM 1975): X ray, ultraviolet light (UV) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG). Screening, also previously described, was done by plating on agar followed by picking colonies into aliquots of liquid medium and, after sufficient proliferation (2–10 days, depending on the size of the colonies picked), examining the cells under a dissecting microscope for evidence of defective motility. The mutant phenotypes detected so far were readily distinguishable from wild type with low magnification, as shown in Table 1. Spontaneous mutation, though infrequent, nevertheless made a significant contribution.

Genetic methods: The procedures employed, briefly described earlier (HIMES and BEAM 1975), depend upon recognizing appropriate life cycle stages. *C. cohnii* grows vegetatively in cysts, which divide into 2, 4 or 8 daughter cells. In liquid medium, these emerge as flagellated swarmers; on agar, as nonmotile autospores, both of which in turn encyst to repeat the vegetative division cycle. Some of the swarmers also play the role of gametes, fusion between any two of them producing a larger swarmer, which soon encysts and, like a vegetative cyst produces 2, 4 or 8 progeny. At present there is no morphological basis for distinguishing between a vegetative mitotic cyst and a zygotic one. *C. cohnii* is homothallic; mating mixtures of two mutants thus contain zygotes of intraclonal origin (homozygous zygotes) as well as the desired doubly heterozygous ones. Fortunately, at least with our motility mutants, complementation was rapid, in some cases within 6 hr, in most cases within 24 hr, producing large, normally swimming cells, which were easily distinguished from the background cells of the parental mutant types.

These fast-swimming zygotes were isolated with a micromanipulator on agar, where they encyst and undergo division. Zygotes usually complete division within 24 hr. As soon as the cyst wall softens, the products can be separated, isolated and placed in liquid. If this is done promptly, "post meiotic" mitoses will not have occurred, and the resulting 2, 4 and 8 clones will represent the immediate products of the zygotic cyst. Much of the tetrad data to be presented from such prompt clonings are marred by cell death, such that occasionally only one of the cosegregants is represented in the segregation. These incomplete tetrads are presented as "random segregants" in Tables 2, 3, 6, 8. Those cysts from which representatives of both complementary segregants (co-segregants) were recovered are tabulated as reciprocal ditypes.

If zygotic products are not separated soon after the softening of the cyst wall, growth and mitoses will occur, producing colonies of more than the original number of cells. Since these mitoses are not always synchronous, co-segregant ratios may become distorted from the original 1 : 1. Cell death also occurs here to further modify the final ratio, such that a 10-cell post zygotic colony from which 9 clones were recovered might present a ratio of 6 : 3. In many crosses, post-meiotic divisions were encouraged rather than avoided for two reasons. First, cloning after these divisions occur affords an opportunity to monitor "postmeiotic" segregation. Second, allowing the segregants to proliferate prior to cloning reduces the chance that cell death will

result in the total loss of a co-segregant from the "tetrad"* One unfortunate result of this strategy is that the observed frequency of two-celled segregations represents a minimal value only.

When the segregant clones grow to adequate cell density (5–10 days), their phenotypes may be established by microscopy and their genotypes determined by complementation testing. Phenotypes alone often provide little information, in part because of epistatic effects, as will be explained, but largely because many of the nonallelic mutant genes confer identical impairments (Table 1). Complementation testing was performed by mixing 0.1 ml aliquots of a log-phase culture (10^5–10^6 cells/ml) with a similar sample of each of the singly marked "tester" strains, and observing the mixtures over a period of 2–3 days for the appearance of normal, rapidly swimming cells—the results of complementations. If the segregant carries the normal allele of the tester, all of whose other relevant genes are normal, complementation will occur. Conversely, failure to complement indicates either failure to mate, or that the new clone and the tester share a mutant allele. Since all mutants show homotypic fusions, and none are totally sterile, a mutant's "sterility" with a particular tester is at present operationally indistinguishable from allelism.

Some fast swimming cells, presumed to be zygotes, were isolated, the progeny of which proved to be all of the same mutant genotype. This result would be expected only if homozygous zygotes or mitotic cysts had somehow been isolated. Since, however, the genotypes of the progeny from these presumed zygotes were predominantly recombinant (see Table 3), these "zygotes" cannot represent parental cells isolated by mistake. A possible interpretation of these findings is that, following normal heterotypic mating and complementation, at which time we normally isolate zygotes, some reduction divisions had already occurred. We propose that some genotypically mutant segregants maintained the wild-type motility of the zygotes from which they came long enough to be observed and isolated in the belief that they were zygotes. We suggest the operation of a kind of phenotypic lag (TATUM 1946, WRIGHT and MOYER 1968). Lag in the expression of mutant phenotypes would be undetected in our usual procedure in which the immediate phenotypes of the segregants cannot be expressed, because *C. cohnii* does not develop flagella on agar, and in which segregants are analyzed only long after growth and proliferation have given new genotypes opportunity for expression. Unfortunately, as previously stated, there is as yet no detectable difference between a mitotic and a zygotic cyst that would otherwise permit identification of the fast swimming "nonzygotes," other than in terms of the genetic character of their division products.

Data from these "mitotic cysts" are included as random segregants in Tables 2, 3, 6 and 8, along with those tetrads from which only one segregant was recovered.

RESULTS

It can be seen in Table 1 that we have now eight nonallelic motility lesions in the Woods Hole strain, as well as nine obtained from newly isolated representatives of *C. cohnii*. One mutant from the Trenton, Maine strain reverted to wild type shortly after its identification as a new genotype, precluding further study. The recurrence of particular lesions has not been noted except for those obtained from different mutagenic treatments, which show (Table 1) that mutants of genes *a*, *b*, and *m* have occurred a number of times. In only one case has the same lesion been found in different isolates; this was the spontaneous occurrence of a double lesion at the *a* and *b* loci in PS-1, identical to the spontaneous double lesion in WH-d previously reported (HIMES and BEAM 1975).

A point should be made about our Puget Sound strain and its sexual compatibility with WH-d. We have isolated several other strains of small coccoid dinoflagellates that are indistinguishable grossly and in Feulgen stained prepara-

* The word "tetrad" here refers to the products of a segregation, whether from 2-, 4- or 8-celled cysts.

tions, as well as in nutritional requirement, from *C. cohnii*, but which are incompatible with those studied here. These came from Puerto Rico (3 strains), Honduras, California, Western Long Island, Florida, Taiwan, and Hong Kong (1 strain each). Since Puget Sound, Washington, provides at least as much occasion for genetic isolation as many of the other sources, it is perhaps prudent to hold in reserve the possibility that either WH-d, currently the most widely employed strain of *C. cohnii*, or another Northeastern representative might have been inadvertantly introduced into this locale.

Although no study has yet been undertaken of mutation rates, forward or reverse, with or without mutagenesis, our overall impression is that, following mutagenesis, 100–1000 colonies must be scored for each mutant found. This frequency, 10^{-2}–10^{-3} per survivor, agrees approximately with the findings of ROBERTS *et al.* (1974) for other induced mutations in this organism. Spontaneous reversion is more variable. In some cases, it was so frequent that the mutant was useless and was either lost or discarded; with other mutants reversion occurs only once in several transfers (~50 cell generations); in some it has never occurred.

Growth rate of most mutants is approximately normal (generation time is 8 hr) except for the paralyzed and flagellaless ones. These settle to the bottom and grow slowly, probably because of crowding. When agitated gently in a New Brunswick tissue culture roller drum, these mutants grow a little more rapidly. This gentle agitation also facilitates matings of nonmotile mutants, which would make few cell-to-cell contacts without it.

Whereas none of the defects have been studied to determine the cause of the phenotypic effect, some intergenic effects have been observed in segregants carrying more than one mutant gene. Epistasis was found in the following order: *e*>*d*, *f*,*o*>*h*>*k*>*a*, *b*, *g*, *i*, *j*, *m*, *n*, *p*, *q*. Thus when *e* is present with any other markers, the cell is phenotypically *e*, which is reasonable since this lesion is the most severe, the cells possessing no observable flagella (light microscopy, see Table 1); *d*, *f* and/or *o*, which confer paralysis to the flagella, similarly prevail over the determinants that produce alone less extensive impairments. No phenotypic distinction can be made between members of two groups of nonallelic genes, and no additivity is observed when two or more of the same group are present simultaneously. This is true of the group, *d*, *f* and *o*, as well as the slow swimming mutants, *a*, *b*, *g*, *i*, *j*, *n*, *m*, *p* and *q*. Only by complementation testing can members of these groups be distinguished. Additivity has been observed only when *k* is present with any of the genes conferring the slow swimming phenotype. In these cases, the speed of the circular swimming of *k* is greatly reduced.

In order to study the segregation of as many genes as possible, multifactor crosses were used whenever convenient. Stocks for such crosses were prepared only after the markers had been shown to segregate and recombine in two-factor crosses. Table 2 shows one such cross. Since phenotypic distinctions are virtually useless, at noted above, all segregants chosen for use in the preparation of crosses were checked repeatedly in complementation tests with testers, the identity of which was also repeatedly challenged. The results of a representative 7-factor

TABLE 2

Analysis of a two-factor cross

Parents
$b+ \times +j$
Products

Reciprocal ditypes						Random segregants							
Parental ditype (PD) $b+/+j$			Nonparental ditype (NPD) $bi/++$			Parental (P)				Nonparental (NP)			
Genotype ratio			Genotype ratio			$b+$		$+j$		bi		$++$	
1:1	(2/2)*	5†	1:1	(2/2)*	4†	(2/1)*	1†	(2/1)*	2†	(2/1)*	2†	(2/1)*	0†
2:2	(4/4)	3	2:2	(4/4)	5	(4/1–3)	9	(4/1–4)	10	(4/1–3)	10	(4/1–3)	12
2:1	(4/3)	6	2:1	(4/3)	1	(8/3–4)	2	(8/4)	1	(8/4)	1	(8/1–8)	0
1:2	(4/3)	4	1:2	(4/3)	1		—		—		—		—
1:1	(4/2)	4	1:1	(4/2)	1		12		13		13		12
		—	2:3	(8/5)	1								
		2			—	Total	P:NP						
					13		47:38						

* Number of cells produced and isolated/number survived and tested.
† Number of incidences.

TABLE 3

Parents of seven factor cross

$a{+}{+}g{+}{+}j \times {+}be{+}hi{+}$

Products				
Reciprocal ditypes	Genotype ratio		Random segregants	
$+b{+}{+}{+}{+}{+}$ / $a{+}egh ij$	1:1	(4/2)*	$+b{+}{+}{+}{+}{+}$	(4/1)*
$+{+}e{+}hi{+}$ / $ab{+}g{+}{+}j$	1:1	(8/2)	$abe{+}{+}{+}{+}$	(8/4)
$+{+}e{+}hi{+}$ / $ab{+}g{+}{+}j$	2:1	(4/3)	$a{+}{+}{+}{+}i{+}$	(4/2)
$+{+}eg{+}i{+}$ / $ab{+}{+}h{+}j$	1:4	(6/5)	$+{+}{+}g{+}{+}j$	(2/1)
$+{+}{+}g{+}{+}j$ / $abe{+}hi{+}$	2:2	(4/4)	$ab{+}{+}hi{+}$	(2/1)
$ab{+}{+}hi{+}$ / $+{+}eg{+}{+}j$	4:2	(12/6)	$ab{+}gh{+}{+}$	(2/2)†
$ab{+}gh{+}{+}$ / $+{+}e{+}{+}ij$	2:1	(4/3)	$+be{+}hij$	(4/3)‡
$+{+}{+}{+}{+}{+}{+}j$ / $abeghi{+}$	2:2	(4/4)	$a{+}{+}{+}{+}{+}{+}$	(4/2)
$+{+}{+}{+}{+}{+}{+}j$ / $abeghi{+}$	2:1	(4/3)	$a{+}{+}{+}h{+}{+}$	(2/1)
$+b{+}g{+}{+}{+}$ / $a{+}e{+}hij$	2:1	(4/3)	$a{+}{+}{+}h{+}{+}$	(4/2)
$+b{+}{+}{+}{+}j$ / $a{+}eghi{+}$	1:1	(4/2)	$a{+}{+}{+}hi{+}$	(3/2)‡
$+{+}eg{+}{+}{+}$ / $ab{+}{+}hij$	1:3	(6/4)	$a{+}{+}ghij$	(6/4)
$+{+}e{+}h{+}{+}$ / $ab{+}g{+}ij$	2:1	(9/3)	$+b{+}{+}h{+}{+}$	(4/1)
$+{+}e{+}{+}{+}j$ / $ab{+}ghi{+}$	1:1	(4/2)	$+b{+}ghi{+}$	(4/2)
$+{+}{+}g{+}i{+}$ / $abe{+}h{+}j$	1:1	(4/2)		
$+{+}{+}{+}hi{+}$ / $abeg{+}{+}j$	2:1	(4/3)		
$a{+}{+}{+}{+}ij$ / $+ebgh{+}{+}$	4:3	(8/7)		
$a{+}{+}{+}h{+}j$ / $+beg{+}i{+}$	2:1	(4/3)		
$+b{+}{+}hi{+}$ / $a{+}eg{+}{+}j$	2:2	(4/4)		
$+b{+}{+}hi{+}$ / $a{+}eg{+}{+}j$	3:1	(8/4)		
$+{+}e{+}h{+}j$ / $ab{+}g{+}i{+}$	2:1	(6/3)		
$+{+}{+}g{+}ij$ / $abe{+}h{+}{+}$	1:1	(2/2)		
$a{+}e{+}{+}i{+}$ / $+b{+}gh{+}j$	1:1	(4/2)		
$ab{+}g{+}{+}{+}$ / $+{+}e{+}hij$	4:3	(8/7)		
$abeghij$ / $+{+}{+}{+}{+}{+}{+}{+}$	3:4	(8/7)		

* Number of cells produced and isolated/number survived and tested.
† Mitotic cyst.
‡ Probable mitotic cyst.

cross are given in Table 3. The parental strains, $a{+}{+}g{+}{+}j$ and $+be{+}hi{+}$, were derived from the following sequence of crosses:

1) $g{+} \times {+}h \rightarrow (gh$ and $+{+})$ as well as $(g{+}$ and $+h)$
2) $i{+} \times {+}j \rightarrow (ij$ and $+{+})$ as well as $(i{+}$ and $+j)$
3) $gh{+}{+} \times {+}{+}ij \rightarrow (ghij$ and $+{+}{+}{+})$ and others, i.e., $(g{+}{+}{+}$ and $+hij)$
4) $abcd{+} \times {+}{+}{+}{+}e \rightarrow (ab{+}{+}e$ and $+{+}cd{+})$ and others*
5) $ab{+}{+}e{+}{+}{+}{+} \times {+}{+}{+}{+}{+}ghij \rightarrow (a{+}{+}g{+}{+}j$ and $+be{+}hi{+})$ and others

The number of cells in the cyst (or in the post-zygotic microcolony) does not often correspond to the number of clones finally established from the segregation, a result of apparently random cell death, as mentioned earlier. This information is included in Tables 2 and 3. It can be seen, nevertheless, that the distribution

* This cross was reported in HIMES and BEAM 1975.

TABLE 4

Cysts from which all products were recovered

	Meiotic cysts				
	2 celled	4 celled	8 celled	Total	Percent of total cysts
Genotypic ratios	1:1	2:2	4:4		
Number	31*	117	15	163	67
%	19	72	9		
	Mitotic cysts				
	2 celled	4 celled	8 celled	Total	
Genotypic ratios	2:0	4:0	8:0		
Number	40	17	0	57	23
%	70	30	0		
				220	90

* These segregations are presented in Table 7.

of genotypes in reciprocal ditypes is similar to that found among the random segregants, and is in good agreement with our previous findings (HIMES and BEAM 1975), both with respect to the recovery of input genetic information and to the relatively free recombination between the markers studied.

Tables 4 and 5 summarize the analysis of those isolates, both zygotes and mitotic cysts, from which all of the products were recovered, *i.e.*, 244 segregations derived from many crosses. The results are subdivided in terms of the number of cells produced in the division cyst. Ratios presented are of the genotypes of co-segregants without specifying their nature. It can be seen that zygotes usually produce four-cell cysts, while mitotic cysts most frequently contain only two products. The occurrence of two-celled meiotic cysts was reported earlier (HIMES and BEAM 1975) and should be noted here as being a rather common event, even though reduced in apparent frequency by post-zygotic mitoses, as previously explained.

In addition to regular segregations, the meiotic cysts include 24 (about 10%) that are aberrant; these are shown in Table 5. In about half of these, Type 1,

TABLE 5

Unusual meiotic segregations

				Number	Percent of total cysts
Type 1	2 : 1 : 1* (9)†		4 : 3 : 1 (2)	11	4.5
Type 2	2 : 1 (2)	4 : 2 (1)	4 : 3 (1)	11	4.7
	3 : 1 (2)	3 : 2 (3)	5 : 2 (1)		
Type 3	1 : 1 : 1 : 1 (1)		1 : 1 : 3 : 5 (1)	2	0.8
				24	10

* Genotypic ratios.
† Number of incidences is given in parentheses.

one of the expected segregants has been replaced by one that appears wild-type; this probably results from reversion as was reported previously (HIMES and BEAM 1975), where it was shown that such apparent wild-type clones contain cells of the expected segregant, as well as wild-type ones. Type 2, in which the ratio, but not the nature of the segregants, is disturbed, we believe to result from asynchronous mitoses following the reduction division. Finally, in Type 3, there are two occurrences of what are technically tetratypes. There are several possible explanations for these segregations in addition to the classical meiotic one: a secondary fusion of products and reduction following the initial reduction division, or a mitotic division of the zygote followed by two independent reduction divisions. The 5 : 3 : 1 : 1 array of four different genotypes appears further perturbed by asynchronous post reductional mitoses. We are unable at present to distinguish among the possible interpretations of these rare events.

The genetic analysis of 16 genes requires examination of the 120 possible pairwise segregations. In the present study, representing over 850 segregations from over 30 different crosses, we found that each gene shows recombination with the others. Thus, if linkage is present, it is in no case complete, as in the yeast *Saccharomycodes ludwigii* (YAMAZAKI, OHARA and OSHIMA 1975). For only 43 pairs are the data sufficient for the estimation of linkage. These segregations are presented in Table 6, where ditypes from two-celled cysts are listed separately. It can be seen that these ditypes indicate no clear differences from the others. The two-celled ditypes from all the crosses are presented in Table 7. Most of the segregations in Table 6 show no suggestion of a departure from a 1:1 ratio of parental (P) to recombinant (NP) types. For only one pair of genes, *b* and *j*, do the summary data show a significant excess of parentals. In three others— *b* and *e*, *a* and *e*, *e* and *m*—the ratios were, however, sufficiently skewed to warrant more detailed scrutiny, and when individual crosses involving these pairs were separately examined, heterogeneity was revealed, as shown in Table 8. With respect to *b* and *e*, the cross *a b c d* × *e* shows a significant excess of parentals, as was previously reported (HIMES and BEAM 1975). Later crosses involving *b* and *e*, however, gave equal numbers of parentals and recombinants. Similar results were found with two other gene pairs, *a* and *e* and *e* and *m*, in which one cross showed evidence of linkage, whereas others did not. The fourth gene pair, *b* and *j*, showed in all crosses a significant probability of linkage, but only in the reciprocal ditype data; the random segregants showed equal numbers of parentals and recombinants.

Similar but less significant heterogeneity was noticed in the data for two other gene pairs. In one pair, there was a marginally significant excess of parentals in one cross that was not apparent in the summary data; in another a similar excess of nonparentals was observed in one cross. In both cases $p \approx .03$, but the numbers were low. In no case did the summary 1 : 1 data contain balanced, significant excesses of both parental and nonparental segregations in different crosses.

In an organism such as *C. cohnii* in which gametes and zygotes appear to occur in a wide range of DNA content and chromosome number (BEAM and HIMES

TABLE 6

Pairwise segregations from all crosses

Gene pair	All reciprocal ditypes PD:NPD	Reciprocal ditypes from two-celled cysts PD:NPD‡	Random segregants P:NP	Total P:NP
a and *b*	95:116	4:7	67:45	162:161
a and *c*	57:53	4:3	—	57:53
a and *d*	82:75	5:5	43:59	125:134
a and *e*	71:79	3:6	65:45	136:124
a and *g*	26:22	0:2	8:13	34:35
a and *h*	23:23	1:1	8:13	31:36
a and *i*	26:20	2:0	12:9	38:29
a and *j*	20:26	0:2	9:12	29:38
a and *k*	26:24	1:1	41:37	67:61
a and *m*	25:24	1:1	44:37	69:71
b and *c*	58:52	4:3	—	58:52
b and *d*	89:80	12:3	60:49	139:129
b and *e*	101:73*	7:5	72:74	173:147
b and *g*	19:27	1:1	11:10	30:37
b and *h*	23:23	2:0	11:10	34:33
b and *i*	22:24	1:1	10:11	32:35
b and *j*	56:32†	6:5	52:44	108:76*
b and *k*	26:26	1:1	43:37	69:73
b and *m*	27:23	1:1	49:45	76:68
c and *d*	54:56	6:2	—	54:56
c and *e*	23:25	3:3	—	23:25
d and *e*	43:54	6:6	46:59	89:113
d and *k*	27:21	1:1	43:39	70:60
d and *m*	21:29	1:1	39:30	60:59
e and *g*	24:22	2:0	10:11	34:33
e and *h*	24:22	1:1	9:12	33:34
e and *i*	23:23	0:2	9:12	32:35
e and *j*	24:23	2:0	14:22	38:45
e and *k*	19:34	1:1	45:33	64:67
e and *m*	34:20	1:1	54:43	88:63
g and *h*	54:36	1:1	21:28	75:64
g and *i*	47:59	0:2	32:36	79:95
g and *j*	50:56	2:0	28:30	78:86
g and *k*	8:13	—	8:7	16:20
g and *m*	12:9	—	5:10	17:19
h and *i*	43:46	1:1	27:21	70:67
h and *j*	41:44	1:1	25:23	66:67
i and *j*	50:42	0:2	24:34	74:76
i and *k*	8:12	—	6:10	14:22
i and *m*	7:13	—	9:5	16:18
j and *k*	8:12	—	10:4	18:16
j and *m*	7:14	—	15:10	22:24
k and *m*	29:41	0:2	40:44	69:81

* $P < 0.02$.
† $P < 0.01$.
‡ Data derived from 31 two-celled cysts (Table 7).

TABLE 7

Two-celled meiotic cysts from all crosses

Cross	Cosegregants, 1/1
$a \times b$	$++/ab$
$b \times d$	b/d ; $++/bd$
$b \times j$	b/j (5)* ; $++/bj$ (4)*
$b \times e$	b/e
$f \times h$	f/h
$d \times m$	d/m
$be \times d$	be/d ; b/de
$bj \times e$	e/bj
$m \times ko$	o/km
$ac \times bd$	c/abd ; ac/b
$a \times cde$	ae/cd
$b \times cde$	de/bc
$abcd \times e$	bd/ace ; $abcd/e$; abe/cd ; ae/bcd
$aem \times bdk$	$abdemk/++++++$
$abem \times dk$	bkm/ade
$bdehi \times agj$	$dgij/abeh$
$abgh \times deij$	$dgi/abehj$

* The number of incidences, when more than one.

1974), one would expect polyploid meioses and/or postmeiotic segregation. The former should be expressed as irregular segregation and inviability of segregants (ROMAN, PHILLIPS and SANDS 1955). Postmeiotic segregation should produce either clonal heterogeneity or segregation ratios more complex than 1 : 1. Depending on which postmeiotic division produced the segregation, and after how many divisions the cloning was performed, 4-, 8- or 16-celled cysts or

TABLE 8

Genetic analysis of some individual crosses

Gene pair	Cross	Tetrads PD : NPD	Random segregants P : NP*	Total P : NP	
b and *e*	$abcd \times e$	36 : 15	7 : 10	43 : 25	$p < 0.03$
	all other crosses	65 : 58	65 : 64	130 : 122	
a and *e*	$abd \times emk$	1 : 0	15 : 3	16 : 3	$p < 0.003$
	all other crosses	70 : 79	50 : 42	120 : 121	
e and *m*	$bdk \times aem$	21 : 10	22 : 13	43 : 23	$p < 0.03$
	all other crosses	13 : 10	32 : 30	45 : 40	
b and *j*	$b \times j$	22 : 13	25 : 25	47 : 38	$p < 0.3$
	others	33 : 20	26 : 18	59 : 38	$p < 0.3$
	Total *b* and *j* tetrads	55 : 33 $p < 0.02$	52 : 44	108 : 76	$p < 0.03$

* P : NP — parental : nonparental for the two genes under consideration.

colonies should produce a variety of complex genotypic ratios, which have never been observed. They should also include numerous tetratypes, whereas these have appeared only very rarely. If segregation occurred after isolation of the cell, the result would be clonal heterogeneity. This would be detected when such a clone is used in further crosses. For example, if a presumed $a++$ clone contained some $ab+$ cells, it would be undetectable phenotypically and in complementation testing, but, if the clone were used in a cross with $++c$, some of the segregants from an $ab+/++c$ zygote (the $+b+$ genotype) would complement both of the presumed parents ($a++$ and $++c$), necessitating the hypothesis that one of the parental strains contained the *b* gene along with the other lesion. This, though never occurring in the analysis of segregants in the present study, was in fact how the *b* gene was found, as an $ab+$ "contaminant" in an $a++$ clone (HIMES and BEAM 1975).

The inviability of segregants, though extensive in many crosses, is unaccompanied by other evidence of polyploid meiosis, and is no greater than that found when individual vegetative cells are isolated. This may result from micromanipulation or the transfer of cells from solid to liquid medium. Therefore, despite the cytological evidence of the likelihood of polyploidy, *C. cohnii* behaves genetically as though haploid genomes were fusing and separating.

DISCUSSION

The major significance of the present work pertains to the nature of meiosis in *C. cohnii*. The use of five different strains as sources of genetic material has provided no exception to the pattern previously described with WH-d (HIMES and BEAM 1975). Crosses within or between these strains have shown no deviation from the ditype mode of reduction as reported, suggesting that this behavior is characteristic of the species and perhaps of dinoflagellates in general. No one strain appears to be more vigorous or more or less mutable than the others, or in any other way preferable for genetic studies.

Strong evidence that meiosis can be concluded in one division is provided by the considerable number of zygotic cysts which contain only two products. One division produces two cells, which show segregation of input markers, either as parental ditypes or non-parental ditypes, with no loss of information and without "postmeiotic" segregations.

In the majority of zygotic cysts, however, a second nuclear division follows the first to produce four-celled cysts, and occasionally a third produces eight cells. In these, whether or not segregation occurs after the first division must be inferred from genetic studies. The present analysis of over 240 segregations, most of which produced more than two cells, yielded only two tetratypes, and one of these was atypical (*i.e.*, segregant clones showing 5 : 3 : 1 : 1 ratio). Both can be accounted for other than by second-division segregation. Thus, even when two or more divisions occur in a zygotic cyst, or are followed by postzygotic mitoses, only the first appears to be segregational. These results agree with our previous findings (HIMES and BEAM 1975), from which it was argued that, if *C. cohnii* underwent the conventional two meiotic divisions, either every marker

is close to a centromere or crossing over is absent. A case of the latter has been recently reported for the yeast *Saccharomycodes ludmigii* (YAMAZAKI, OHARA and OSHIMA 1975). Strong evidence of linkage and crossing over would, nevertheless, eliminate these possibilities and favor the single division alternative for the 4- and 8-celled zygotic cysts of *C. cohnii*.

It may be meaningless to consider centromere linkage in dinoflagellates, since the chromosomes of these organisms attach to the nuclear membrane rather than to the spindle fibers directly (KUBAI and RIS 1969), and no specific sites of attachment have been demonstrated. Furthermore, the probability that 16 loci at random should be inseparable from such sites seems very low. For these reasons we doubt that centromere linkage is the basis of our findings.

There is some indirect evidence of gene–gene linkage and crossing over in *C. cohnii*. Each of our 16 markers recombines with all of the others. The probability that no two of the sixteen reside on any one chromosome depends on the chromosome number. Over 100 chromosomes have been counted in this organism (ALLEN *et al.* 1975), and we also have noted many cells containing about 100 chromosomes in Feulgen stained preparations; but we have also observed cells with as few as 50. Using either number, it can be calculated* that the probability of each marker being on a different chromosome is extremely small. Thus even without direct evidence, the occurrence of linkage at sufficient distance to permit frequent crossing over is highly likely.

Some direct evidence of linkage and crossing over has also been found. It is, however, unorthodox. Genes *b* and *j* appear linked in the reciprocal ditype data from all crosses, but events producing incomplete tetrads or "mitotic" segregants seem also to produce independent assortment of these markers. Gene *e*, when first isolated and tested, showed linkage with *b*, but in later crosses involving *b* and *e* in recombinant arrays, they assorted independently, as though recombination could "unlink" linked genes.

It is possible that, despite the low *p* values, our numbers are too small for firm conclusions. Nevertheless, if one had to speculate as to the biological significance of these findings, the several prokaryote aspects of the dinoflagellate chromosome provide a basis. If membrane attachment of chromosomes is of importance to their separation in meiosis, and if chromosomes maintain their position for several generations, genes on chromosomes attached to adjacent points might at some times appear to be linked. This argument, of course, presupposes little or no crossing over between the attachment site and the marker. Alternatively, if each chromosome were basically circular, complicated only superficially by successive coilings, and if crossing over entailed breaking the circles, alternate breakage points, sometimes between, sometimes to one side of two linked genes could provide a basis for the observed cross dependent linkage. If such events sometimes had lethal consequences, the absence of linkage in the incomplete tetrads and random segregants of *b* and *j* might also be accounted for.

Whatever the basis for suspension of independent assortment in *C. cohnii*, its behavior is quite different from that of *Saccharomycodes ludwigii* (YAMAZAKI,

* The probability, p, that no two of n genes reside on any one of X chromosomes is given by: $p = \frac{X!}{(X-n)!X^n}$. With n=16 and X=50, p=0.065; with X=100, p=0.282.

OHARA and OSHIMA 1975), where from 22 markers, nine of the 60 gene pairs analyzed displayed absolute linkage; not only were tetratypes very rare, but there was no recombination at all between linked markers, clearly supporting the view that crossing over does not occur in this organism. "Linkage" in *C. cohnii*, in contrast, appears to be always accompanied by a high frequency of recombination, resembling the cross-dependent "false" linkage sometimes observed in Chlamydomonas (GOWANS 1976). Thus, though aberrant, the present evidence of linkage and crossing over is consistent neither with centromere linkage nor absence of crossing over, which otherwise stands in the way of accepting a one-division meiosis in *C. cohnii*, and one in which crossing over, when it occurs, is at the "two-strand" stage.

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