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Cortical morphogenesis in *Paramecium aurelia*: mutants affecting cell shape

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SUMMARY

In order to investigate the importance of the nuclear genotype during morphogenesis of the cortex in *Paramecium aurelia*, a search was made for conditional and unconditional mutants affecting cell shape. Ten different mutants were readily recovered following mutagenesis with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine on stock 51s of syngen 4. The detailed pattern of surface units remained unchanged in these mutants but each one can be described as resembling a normal cell twisted about its long axis. Nine of the mutants complement each other and show no linkage. The remaining mutant, a 'chain former', has conditional expression, and is only recognizable at 35 °C. The penetrance of this mutant is affected by the food organism used. The conditional mutant also interacts with seven of the other mutants in pairwise homozygous combinations so that in these genotypes it can be classified after growth at 25 °C. The mutants are discussed in relation to the search for genic effects on the cortical pattern.

1. INTRODUCTION

The ciliate *Paramecium aurelia* presents a very interesting system for the study of the control of cell morphology. The cortex of this organism includes a precise arrangement of organelles at the cell surface. This comprises a series of longitudinal rows (kineties) of repeating basic unit structures of two types (see Sonneborn (1970) for recent review and references). Most groups of kineties in a cell can be distinguished from one another by the distribution and number of the two types of unit in them. This pattern is accurately reduplicated at cell division (Sonneborn, 1970; T. M. Sonneborn & L. Chen-Shan, unpublished). Certain induced alterations in this pattern will persist and will themselves be reduplicated stably over many fission generations even though their initiation and perpetuation are independent of any nuclear genic change in the cell line (Sonneborn, 1963; Beisson & Sonneborn, 1965). Paramecium is also very amenable to genetic analysis, and the process of autogamy which results in homozygosity at all loci removes one of the major drawbacks of genetic study in diploid organisms (Sonneborn, 1957).

There is therefore particular interest in discovering the extent to which genic differences can alter this cortical pattern, as a means of dissecting the process of

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cell morphogenesis and also as an approach towards understanding the interaction between genic differences and cytotactic phenomena in cells (Sonneborn, 1963). With this objective it is first necessary to examine how best such mutations can be found. Although several genetic effects on cell shape and aspects of the cortical pattern are known in Paramecium (Beisson & Rossignol, 1969; and references in Sonneborn, 1970), at present there is no systematic method for selecting mutations affecting cortical structure. Some cortical changes are correlated with alterations in cell shape or swimming behaviour (Beisson & Sonneborn, 1965). Therefore a number of mutants with altered cell shape were recovered and examined for possible changes in cortical structure. In this paper the suitability of these mutants for the objective stated earlier is examined. These mutants lacked effects on the aspects of cortical structure subject to cytotactic influences and cytoplasmic inheritance, yet indicated that mutation of a number of loci can affect cell shape, and that penetrance of these mutants is related to culture conditions and genetic background. They have also provided a number of useful morphological markers. A preliminary report of this work has been published (Whittle, 1970).

2. MATERIALS AND METHODS

Cells of *P. aurelia* syngen 4 stock 51s were cultured in bacterized baked lettuce and Cerophyl media at 27 °C. Mutagenesis was carried out by exposure of cells to *N*-methyl-*N*-nitro-*N*-nitrosoguanidine at concentrations between 25 and 75 $\mu g/ml$ for 1 h. Following treatment ex-autogamous cells were isolated, cultured and the resulting clones examined for alterations in cell shape and swimming behaviour. It was anticipated that some changes in cortical structure might not affect cell shape but only swimming behaviour as in the case of cells with kineties of reversed polarity (Beisson & Sonneborn, 1965). Selection was deliberately practised for mutant clones which appeared homogeneous in depression culture and had normal fission rate. Some clones were kindly supplied by C. Kung following similar mutagenesis. Details of cortical pattern were examined using the Chatton–Lwoff–Corliss silver impregnation method (Chen-Shan, 1969) and phase contrast or interference phase optics. The recessive temperature-sensitive lethal ts^{111} was used as a genetic marker to indicate whether nuclear behaviour at conjugation and autogamy was normal.

RESULTS

(i) *Recovery and characterization of mutants*

Ex-autogamous clones of the desired shape and swimming phenotypes occurred frequently following mutagenesis. Each variant was out-crossed to a normal cell line and the F1 clone derived cytoplasmically from the normal parent was taken through autogamy. This procedure separated variants due to nuclear genic factors from those due only to cortical or cytoplasmic changes. No persistent effects of the latter class were obtained.

Single gene differences are recognized as 1:1 segregations among exautogamous clones from the F1. Nine variants were found to give stable and distinct differences

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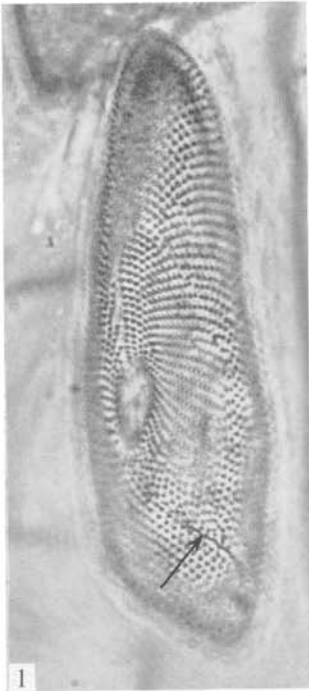

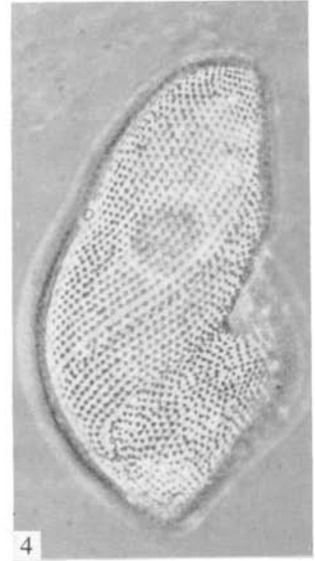

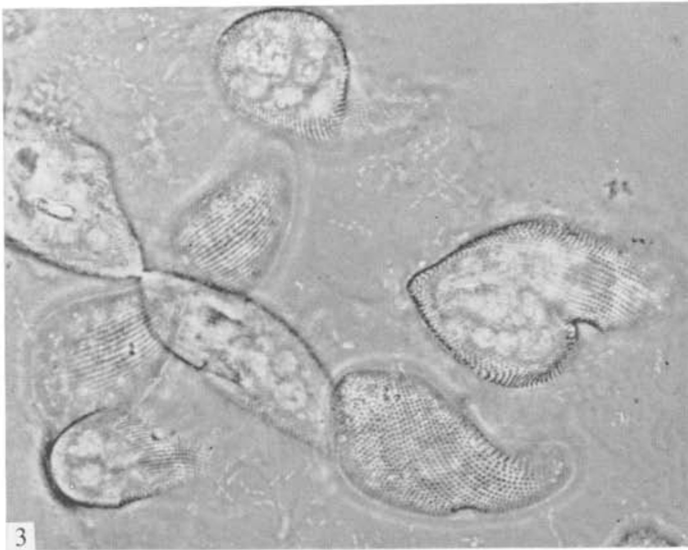

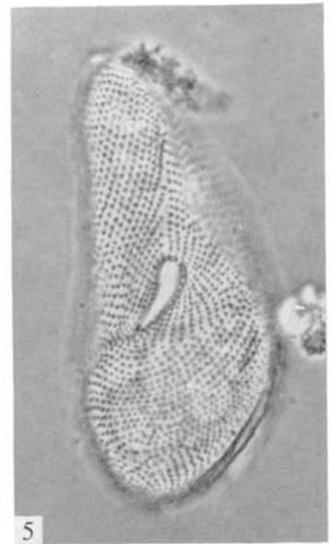

Fig. 1. Mutant 515. Cytoproct line (arrow) at an increased angle to the long axis of the cell. × 400.

Fig. 2. Mutant 65. Right-hand spiralling of kineties. × 400.

Fig. 3. Mutant 56. × 200.

Fig. 4. Mutant 80. × 400.

Fig. 5. Mutant 80. × 400.

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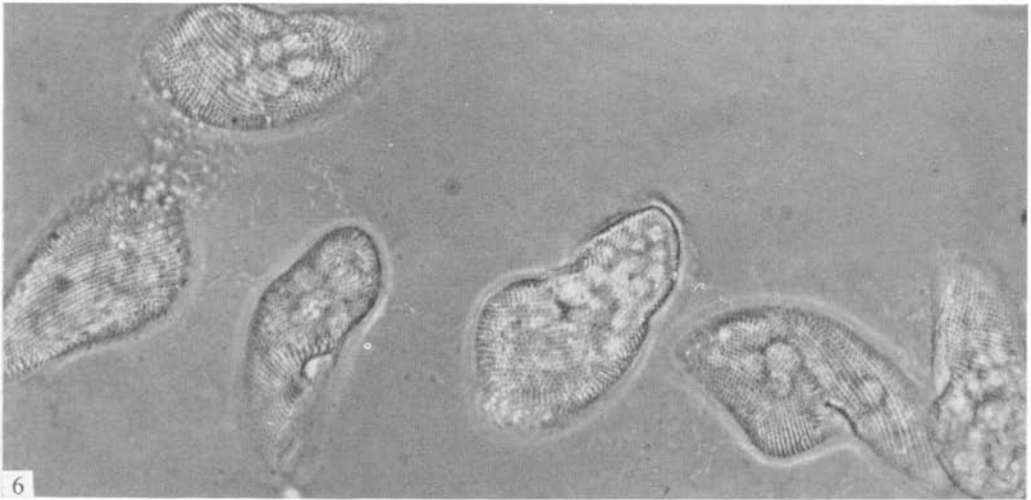

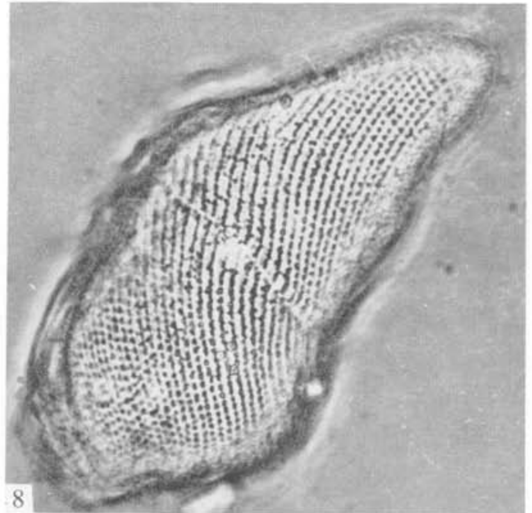

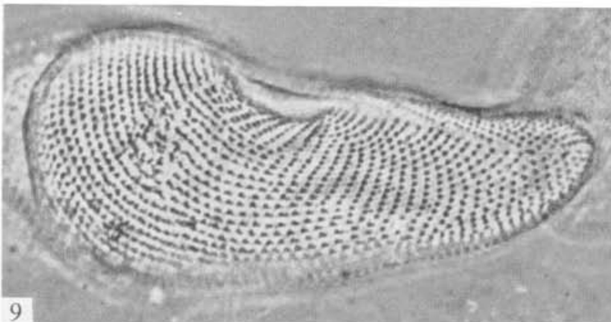

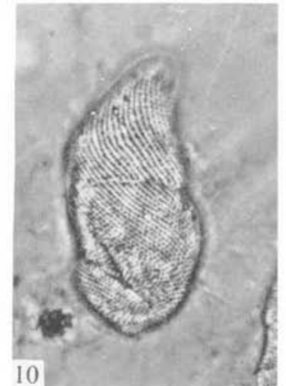

Fig. 6. Mutant 80. × 200.

Fig. 7. 65,515 double homozygote, dividing cell. × 400.

Fig. 8. Mutant 65. Dividing cell. × 400.

Fig. 9. Mutant 80. Curved fission furrow. × 400.

Fig. 10. Mutant 65,515 double homozygote. Dividing cell. × 200.

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Table 1. *Genetic segregation of the variant phenotypes after outcrossing and autogamy*

Variant	Phenotypes of post-autogamous clones: Wild-type	Like original variant
56	44	54
63	113	115
64	47	52
65	97	84
66	108	107
80	146	148
213	111	104
515	92	83
1924	98	110
211*	50	38

For all χ^2 values for a 1:1 expectation, $95\% > P > 10\%$.
* Clonal phenotypes classified after growth at 35 °C.

from wild type and to segregate as single recessive mutations (Table 1). Several of the mutants could be distinguished from one another in depression culture. Mutants 515 and 1924 are phenotypically similar to one another and show more extreme curvature in gross cell outline along the long axis of the cell (Plate 1, fig. 1). Mutant 213 has smaller cells than wild-type. The remaining six mutants may be superficially described by visualizing the appearance of a normal cell subjected to twisting along its long axis (Plate 1, figs. 2–5. Plate 2, fig. 6).

Detailed examination of fixed cells from all mutants revealed that the composition of the various kinety fields was apparently not altered. Although individual kineties described different paths over the cell surface and thus particular kinety fields occupied different areas of the cell surface, yet the distribution of one- and two-cilium units within these fields remained the same. For example, the distinct pattern of two-cilium units in this region (Sonneborn, 1970; T. M. Sonneborn & L. Chen-Shan, unpublished) was not altered in any of the mutants even though the curvature of the kineties varied in different mutants.

In living cultures each mutant appeared to have a uniform cell phenotype, but more heterogeneity in cell shape and cortical pattern was visible in fixed and stained samples, which complicates description of the phenotypes. In clones which had been genotypically homozygous for a certain mutation for a considerable number of fission generations there were occasional cells with kineties of reversed orientation, and also 'monsters' and cells with very disorganized kinety patterns.

Accordingly, cellular phenotypes were examined in clones into which the relevant genotypes had been recently introduced, to distinguish secondary and chance cortical perturbations from the primary effects. Fixation of cells from individual ex-autogamous clones at various fissions following autogamy in a heterozygote has not yet given sufficient satisfactory data on the onset of expression of the mutant

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genes. Mutant phenotypes are classifiable in clones nine or more fissions after autogamy. Dividing cells in clones homozygous for the mutations have shown many examples in which the fission plane remains perpendicular to the long axis of the cell even though the kineties do not intersect it at the normal angles (Plate 2, figs. 7, 8, 10). In contrast some cells were observed with very unusual fission planes (Plate 2, fig. 9).

The twisting of kineties around the cell surface seen in the group of seven twisted mutants mentioned earlier is not of the same handedness in all cells of the same clone and it may be preponderantly different in different clones of the same mutant. Kineties curving to the left counterclockwise in their progression from the anterior pole viewed from that pole define left-handedness. Several independently constituted homozygous clones of each mutant were examined for handedness of the spiralling. Mutant 65 showed a distinct left-handedness in three clones (Plate 1, fig. 2). (61 of 140 cells showed left-handedness, 16 cells of the opposite handedness (Plate 1, fig. 3) and the remainder were straight or equivocal). In mutant 80 the preponderant handedness was opposite in different clones (e.g. 42/46 cells R, 22 of 31 cells L), and in mutant 56 the proportion and direction of bias in handedness also varied from clone to clone.

The clonal homogeneity in cell shape, however superficial, was lost when clones of the mutant became contaminated with certain other micro-organisms. 'Monster' cells arose following failure in cytokinesis, and as judged by this effect all of the mutant stocks had less stable phenotypes than the wild-type 51s.

(ii) *Genetic complementation*

Pairwise crosses between the mutants were made to test for allelism and linkage. Table 2 indicates that of the crosses made there is no linkage or even interaction in the double heterozygotes. One other mutant was found to be allelic to mutant 1924 (personal communication of M. V. Schneller), and so has not been included separately in Tables 1 and 2. Difficulty was experienced in crossing certain pairs of mutants, notably the group containing 56, 65 and 80. In some instances crosses were made between a heterozygote for one mutant and the other homozygote to overcome this difficulty.

When F1 clones were obtained, they yielded ex-autogamous F2 clones in the ratio of one normal clone to three clones of morphological variants. As mentioned earlier some mutants were phenotypically so similar that they could not be separately identified. In all exautogamous clones from crosses between mutants there are clones of more extreme phenotype than either single mutant, which were considered the putative double mutant class though they were only positively identified as such following their behaviour upon outcrossing to wild stocks.

Stable doublet cells arose from some conjugating pairs formed between mutants 56 and 515. This deserves mention in that it occurred under conditions in which persistent deliberate attempts to form doublet cells from normal 51s cells by various techniques had all failed.

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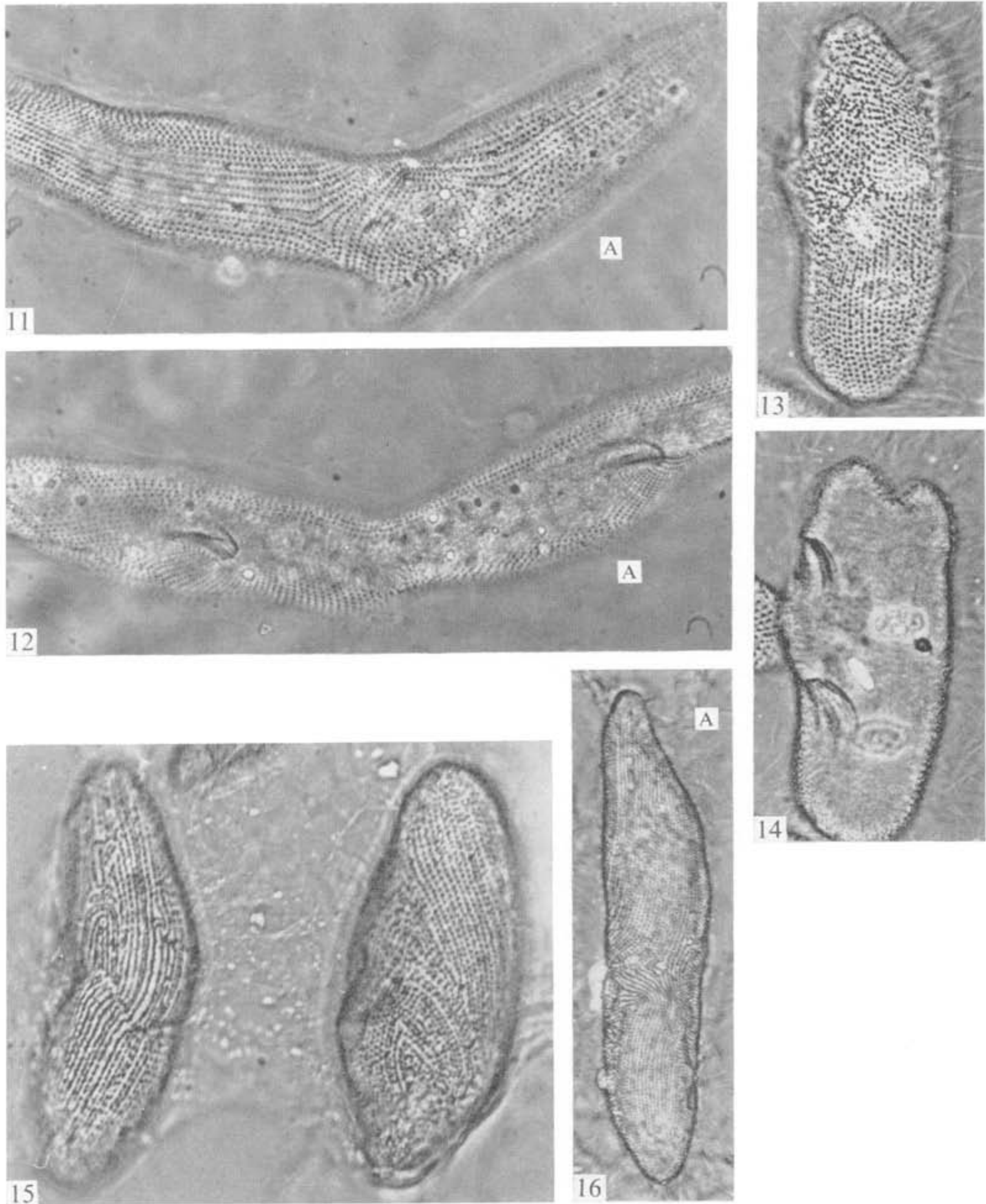

Figs. 11, 12. Mutant 211 grown at 35 °C. × 400.

Figs. 13, 14. Two focal planes of the same cell of genotype 211, 515. × 400.

Fig. 15. Two cells of genotype 211, 515 with gross disorganization of kineties. × 400.

Fig. 16. 211, 515 double homozygote. Disorganized kineties in the equator of cell but no constriction. × 200.

A, anterior end of cell.

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Table 2. *Complementation and linkage tests between mutants*

	1924	515	213	80	66	65	63	56
49	.	+	.	0	.	0	.	0
56	+	+	.	0	.	0	.	.
63	+	+	+	+	+	+	.	.
65	+	+	+	0	+	.	.	.
66	.	+	.	+	.	.	.	.
80	0	+	.	.	.	.	.	.
213	.	+	.	.	.	.	.	.
515	+	.	.	.	.	.	.	.
1924	.	.	.	.	.	.	.	.

+ indicates that the F1 hybrid is wild-type and that the two mutations concerned segregate independently.

0 indicates that repeated attempts to cross the mutants have not yielded true F1's.

(iii) *A conditional morphological mutant*

An alternative approach to the recovery of mutants affecting details of cortical pattern was developed in the following manner. Reduplication of the cortical pattern in *Paramecium* is a very conservative process, and it is possible that direct gene effects on cytotaxis might be lethal. This sort of mutation if it existed could be preserved only if it had conditional expression. A limited search was made for mutations affecting cell shape only at 35 °C but not at 27 °C and an interesting one was found (211).

Mutant 211 is indistinguishable from wild-type at 27 °C, but at 35 °C some 211 cells fail to separate at division and form chains of two cells (Plate 3, fig. 11). Other cells, although twice the length of the normal cell, do not show any indication of a fission furrow further than disordered kineties in that region. In dividing cells many kineties ran uninterrupted from pole to pole with no sign of the gap that normally appears at the fission plane. Chains of cells eventually separated but the products would often possess areas of disorganized kineties related to the abortive division plane of the previous fission.

The clonal penetrance and expressivity of this mutant at 35 °C decreased when cells were washed and grown in clean bacterized medium. Bacterial species contaminating the original culture affected penetrance, and several bacterial strains were isolated from clones of 211 showing high penetrance, but attempts to cross-infect wild-type stocks from 211, or to induce the effect in wild-type stocks fed bacteria or cellular debris from cultures of 211 were totally unsuccessful.

The phenotype of 211 is recessive and the mutant segregated as a single factor in outcrosses (Table 1), though the variable penetrance of 211 affected the segregation ratios.

In the ex-autogamous F2 from a cross between 211 and 515 the phenotype characteristic of 211 was recognizable in many clones of 515 when grown at 25 °C (Table 3). When one of these clones, (C), was outcrossed to wild-type, the F1 was normal but again three phenotypes were recognized in the F2: normal clones, clones resembling 515, and clones in which effects similar to those of 211 were

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Table 3. *Crosses between 211 and 515*

Cross	Phenotypes of ex-autogamous clones at 27 °C			P values for χ^2 tests	
		Mutant			
	Wild-type	Like 515	Like 515 and also chain forming	1:1 wild-type	2:1:1 phenotypic classes
(*a*) 211 × 515	47	42	14*	95% > P > 10%	P < 0·1%
(*b*) C × wild-type	38	23	11	95% > P > 10%	95% > P > 10%
(*c*) C × 515	0	27	34	.	.
(*d*) C × 211	74	23	84	5% > P > 1%	.

* One clone taken from this class was designated C and used in crosses (*b*), (*c*) and (*d*).

Table 4. *Crosses between 211 and other morphological mutants*

Cross	Phenotypes of ex-autogamous clones at 27 °C		
		Mutant	
	Wild-type	Like first-parent	Like first parent with 'chain-formers'
49 × 211	24	13	10
56 × 211	24	14	17
65 × 211	29	10	19
80 × 211	49	27	27
213 × 211	44	34	14
1924 × 211	35	28	17

χ^2 for segregation 2:1:1, gives 95% > P > 10% in all segregations except with 213: 5% > P > 1%.

superimposed on the 515 phenotype (Table 3). Crosses of the same clone C to 515 and to 211 were consistent with the hypothesis that clone C was a double mutant 211, 515 (Table 3).

Crosses were also made between mutant 211 and mutants 56, 65, 80, 213 and 1924 with similar results; approximately half of the ex-autogamous clones with the phenotype of the mutant from the latter group also manifest at 27 °C the phenotype of 211, otherwise only seen after growth at 35 °C (Table 4).

4. DISCUSSION

Genic mutations leading to changes in gross cell shape were recovered at high frequency following mutagenesis (for example, three of the mutants were found in a sample of three thousand ex-autogamous clones). Allelism tests so far available have revealed only one case of allelism between two induced mutants. The high

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frequency of occurrence of the mutants affecting cell shape suggests that a variety of genetic lesions in different developmental and metabolic pathways may all lead eventually to the cell assuming an abnormal shape.

Though these investigations have not so far suggested how the abnormal cell shapes have originated in the mutants described, it seems unlikely that they reflect gross changes in the proliferation sequence or the pattern of units over the surface. Unit territories are formed before cytokinesis, but the fission products are initially shorter than the final interfission length. A redistribution of the resources for elongating the unit structures could underlie some of the distorted cell phenotypes seen, though it is known from the amputation experiments of Chen-Shan (1969, 1970) that adjacent kineties cannot 'slide' longitudinally with respect to one another. The cytoproct and cytoproct line are induced at the junction between the posterior left and right ventral kinety fields (Sonneborn, 1963). The relocation of the cytoproct line at an increased angle to the long axis of the cell, seen distinctly in mutants 515 and 1924, may reflect unequal extension of these kinety fields by unit elongation. The chain-forming phenotype of mutant 211 has been found before in *Paramecium* (Stocking, 1915; Maly, 1958; Sonneborn, 1970). The putative fission line is incomplete in some cells and not all kineties are divided to anterior and posterior sections. Disorganization of kineties is greatest where the fission furrow is least apparent and many cells attain twice the normal length and show normal proter and opisthe structures except at the junction between them. The occasional chain of four cells clearly shows that whatever mechanism specifies local 'position' to cortical areas, therefore determining the sites of unit proliferation, acts autonomously in the two 'halves' of the long cell in the next cycle of proliferation.

Certain bacterial species (Keim & Hanson, 1964) and nutrients (Tartar, 1954) are known to induce morphological changes in *Paramecium*. In the present example the food organism effect on 211 is genotype-dependent like the situation described by Keim & Hanson (1964), but can also be mimicked by all the recessive mutants from the group of ten that have been examined in homozygous combination. The range of genotypes (see Table 4) with which 211 interacts to reveal the chain-forming phenotype at the permissive temperature (27 °C) suggests that some general threshold effect relating to the 211 locus is operating; that many different disturbances to cell shape can reveal the presence of the 211 mutation at 27 °C. It should be pointed out that in none of the other double homozygote combinations (see Table 2 for those constructed) was a chain-former phenotype observed. Examination of the effect of various fractions of the various bacterial species on mutant 211 grown in axenic culture may indicate the direction in which the interaction between 211, and for instance mutant 515, may best be studied. Precisely because of the conservative mode of cortical replication in *Paramecium*, one might expect genetically determined upsets in cortical pattern, once initiated, to be compounded over fission generations, resulting in at least some sublines of the clone becoming increasingly more distorted in their cortical features. Hanson (1962) has described the process of loss or repair of gullet structures following induced

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damage that may continue for many fission generations. Siegel (1970) has recently advanced the idea that alterations in cytotactically determined organelles such as the gullet, can give rise to intra-clonal heterogeneity over a number of fissions. Occasionally abnormal cells and 'monsters' also arise in wild-type clones. Beisson & Rossignol (1969) have described a morphological mutant, m, in which the proter is less abnormal than the opisthe, and the heterogeneity within clones of several of the mutants recovered in this study, particularly 56, 66 and 80 increases with clonal age. Together these facts suggest that the interesting clones to examine after mutagenesis would be those in which cell shape was abnormal but in which clonal heterogeneity also increased with each fission generation. The identification of genetic factors affecting the cortex by the appearance of cumulative abnormality in clones presents other technical problems. Aberrant cell types may lead to upsets in cytokinesis, feeding and loss of the clone. This suggests that such genic factors could be manipulated in crosses and kept as stocks easier if their expression was conditional, for example only expressed at an elevated growth temperature. In addition, the onset of expression of each mutant could then be better studied by inducing the phenotype in a clone of cells by temperature change rather than by examining individual ex-autogamous clones at stages when perforce there are few cells in each clone.

Microtubules feature importantly in cortical structures and also in the mitotic spindle of the micronucleus. Thus a mutation affecting either a subunit common to both structures or a common step in assembly, would lead to the loss both of structural integrity in the cortex and of the micronucleus as the clone grew. If it exists, this class of mutant could also be preserved only if it had conditional expression.

Response to selection within a clone for a cortical feature implies a cytotactic or cytoplasmic basis for the perpetuation of the effect rather than a genic cause. During the investigation of the basis of the phenotype of 211, selection was practised within a clone for more and less extreme cellular phenotypes over a number of fission generations without success; a finding consistent with a genic rather than a cytotactic basis for this phenotype. Isopropyl-*N*-phenyl carbamate interferes with the development of microtubule structure in plants (Helper & Jackson, 1969), and the sulphydryl compounds dithiothreitol and dithioerythritol both act as protective agents for sulphydryl groups (Cleland, 1964). Normal cells were exposed to each of these compounds at sublethal concentrations with the aim of inducing alternative cortical arrangements that might be stably perpetuated but changes were recorded neither in gross clonal appearance nor in the silver line pattern.

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