ANALYSIS OF THE HERITABILITY OF CORTICAL INVERSIONS THROUGH SEXUAL EXCHANGE IN *Paramecium tetraurelia*

A Senior Scholars Thesis

by

REBECCA KANG CROSS

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

May 2012

Major: Molecular and Cell Biology

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Approved by:

Research Advisor: Karl Aufderheide
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ABSTRACT

Analysis of the Heritability of Cortical Inversions through Sexual Exchange in *Paramecium tetraurelia*. (May 2012)

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Paramecium tetraurelia is a large, single-celled, ciliated protist. Short cell cycle times (4.5-5 hours) and manipulable Mendelian genetics have made it an attractive research species, particularly for developmental genetics investigations. A genetic cross between cells with inverted ciliary rows and cells with normal cortexes was performed to determine the heritability of cortical inversions through sexual exchange in *P. tetraurelia.* A nuclear gene, *nd6*, which confers trichocyst nondischarge, was used in the cross to demonstrate a Mendelian inheritance pattern. Quantitative scoring of cortical phenotypes, including the location and size of the inversion, and the total number of ciliary rows was performed for the P_1, F_1, and F_2 generations.

The *nd6* mutation was inherited in a typical Mendelian pattern. The inverted ciliary rows were inherited only through the progeny of the conjugant originally possessing the inversion and were not transferred to the other partner of the cross. Inheritance of the

cortical inversion was stable in each generation; in no instances did F_1 and F_2 lines with inverted rows spontaneously lose them, nor did F_1 and F_2 lines with normal cortexes spontaneously generate inverted cortical rows. These results demonstrate that cortical units follow a non-Mendelian inheritance pattern that is consistent with the phenomenon of structural inheritance, or directed assembly.

DEDICATION

This research is dedicated to my wonderful parents, who have encouraged me to realize and pursue my dreams to the fullest degree.

ACKNOWLEDGMENTS

I offer my greatest thanks and gratitude to my supervisor, Dr. Karl Aufderheide, who has patiently guided me throughout this project. His instruction and correction, while allowing me the opportunity to work in my own way, has made this project immensely rewarding and has enabled me to realize fully my capabilities. Without Dr. Aufderheide's support and endless use of red pen, I would not have been able to complete this project.

NOMENCLATURE

A	Anterior
P	Posterior
R	Right
L	Left
kf	Kinetodesmal Fiber
bb	Basal Body
wj	Wide Juncture
nj	Narrow Juncture
I	Inverted Row
N	Normal Row
R	Rows to Inversion
T	Total Rows

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CHAPTER I

INTRODUCTION

Background

The eukaryotic protozoan *Paramecium tetraurelia* is an oblong, ciliated, single celled organism about 150 μm long and 30 μm wide and has a number of features which make them an attractive research species, including short cell cycle times (4.5–5 hours) and manipulable Mendelian genetics (Sonneborn 1975; Sonneborn 1970). Their large cell size allows relative ease in handling and culture (Sonneborn 1970). Cells contain a large, transcriptionally active, polyploid macronucleus which determines most of the cellular phenotype, and two diploid micronuclei that are the source of genomic material. *P. tetraurelia* is capable of both sexual exchange and autogamy, making it useful for genetic manipulation (Sonneborn 1975).

The cortex of *P. tetraurelia* displays an elaborate array of surface-associated cytoskeletal and membranous structures. About 1–2 μm thick, the cortex is a three-dimensional complex organized around the 3,000 or so somatic ciliary basal bodies of each cell (Sonneborn 1962). Cilia are not haphazardly placed, but are precisely arranged into 70–80 longitudinal rows covering the entire cell surface (Sonneborn 1970; Jerka-Dziadosz and Beisson 1990).

This thesis follows the style of Genetics.

The ciliary rows can be resolved into repeated smaller entities called cortical units, each of which shows anteroposterior polarity, and left-right asymmetry. Each cortical unit consists of one or two ciliary basal bodies and associated structures including a kinetodesmal fiber and a number of microtubular and membranous components (Jerka-Dziadosz and Beisson 1990). The axes of the units in each row have identical orientation, and the local organization of each row normally reflects the cell's global A-P and L-R axes, enabling one to determine the axes of the whole cell by examination of any portion of the cortex (Aufderheide *et al*. 1980).

The set of ciliary rows and their cortical units on the cell surface must be duplicated each cell cycle in order to ensure a proper number and arrangement of cilia in the daughter cells of a division. Cortical unit proliferation begins with the subsurface assembly of a new basal body to the immediate anterior of, and at a right angle to, an existing basal body. This new basal body then moves anteriorly as it rotates on its axis towards the cell surface, into the same orientation as its "parent." Each new basal body then acquires its own associated structures to become a new cortical unit (Sperling *et al.* 1991; Beisson *et al.* 2010). While cortical morphogenesis is the result of typical gene expression, in which transcripts from the macronucleus specify the proteins used to build new cortical structures, an epigenetic process, called cytotaxis, or directed assembly, also plays a role in morphogenesis. The existing 3-dimensional structure of the cortex appears to influence the formation and propagation of cortical units and ciliary rows (Sonneborn 1970; Aufderheide *et al.* 1980).

Beisson and Sonneborn first described cortical inversions in 1964. Inversions involve the 180˚ planar rotation of a portion of the cortex to the cell's anterior-posterior axis. This results in a reversal in the polarity and asymmetry in the region of the inverted cortex (Sonneborn 1970). For example, instead of pointing toward the anterior of the cell, as is typical in the normally oriented cortex, the kinetodesmal fibers of inverted cortical rows point to the posterior of the cell. All structures are normally organized within the local context of the cortical unit, but the orientation of the entire unit is inverted (Aufderheide *et al*. 1980). The cilia in inverted rows beat in a direction opposite to that of normally-oriented cilia, causing the cell to swim in a characteristic "twisty" swimming pattern (Tamm *et al.* 1975; Beisson and Sonneborn 1965). This change in swimming behavior can be easily discerned by observations of living cells with low power light microscopy. Inverted rows are structurally stable and the inversion is propagated to daughter cells during cell proliferation. In inverted regions of the cortex, and there only, new basal bodies assemble to the local anterior of existing basal bodies (not to the cell's anterior), producing an inverted orientation of the axes of new ciliary units (Tamm *et al.* 1975; Beisson and Sonneborn 1965). As a result, inverted ciliary rows are propagated, because the existing ciliary units direct the assembly of new cortical units with the same asymmetric and polar pattern as themselves. This indicates that positioning of new cell cortex components may be influenced by preexisting components (Sonneborn 1962). Sonneborn indicated that the genetic basis for the propagation of inversion was not in the macronucleus, micronuclei, or cytoplasm, but in the cortical structure itself (Preer 1997). The heritability and maintenance of the inverted region of the cortex, generated and

sustained in the absence of detectable nuclear change, demonstrates the phenomenon of epigenetic cytotaxis or directed assembly (Grimes and Aufderheide 1991; Aufderheide *et al.* 1980).

Trichocysts are regulated exocytotic organelles that are distributed regularly along the surface of *Paramecium*. Before exocytosis, the trichocyst is a membrane-bounded organelle that looks like a "carrot with a golf tee" attached to the end, and is 5 μm long. They are associated with the plasma membrane at insertion or docking sites on the anterior-posterior boundaries between cortical units in the surface. When the cell is stimulated by certain chemicals or physical treatments, exocytosis of the trichocysts is triggered and they are explosively released from the cell in about 1 millisecond. The 5 μm "carrot with a golf tee" is converted into a 25 μm spear-like shape (Pollack 1974). About 40 or 50 Mendelian loci have been identified which contribute to trichocyst assembly or discharge at various developmental stages. One such mutation, *nd6,* results in fully assembled and cortically-positioned trichocysts which cannot discharge. This gene has been particularly useful in genetic studies as a marker for Mendelian inheritance patterns (Grimes and Aufderheide 1991; Lefort-Tran *et al*. 1981).

Objective

We hypothesized that, if the inversion and its propagation during cell division are the result of a structural inheritance system, and not regulated by nuclear genes, then an inversion would be inherited in a non-Mendelian inheritance pattern during sexual exchange is *Paramecia*. We predict that nuclear genes would be inherited according to well-known Mendelian patterns, but that inversions would be inherited only in the linear, structural descendants of the original parental cell of a conjugation through the F_1 and F_2 generations.

Quantitative aspects of the heritability of inversions through sexual exchange were studied. Conjugation between a cell with a normal cortex and a cell with an inversion will be studied to determine if the inheritance of a cortical inversion follows a Mendelian or non-Mendelian pattern. In the cross, a gene with a known Mendelian gene pattern, *nd6*, will also be followed to demonstrate a Mendelian nuclear inheritance pattern against the inheritance of the cortical inversion. The size of the inversion, and its location on the circumference of the cell (its "longitude"), were determined at each sexual generation of the typical Mendelian cross.

A genetic cross between a cell line with inverted ciliary rows and wild-type trichocyts ($nd6^+/nd6^+$), and a cell line with normal cortex and homozygous for the *nd6* allele was performed to determine the pattern of inversion inheritance. The corticotype of the cells, including the size and location of the inversion was quantitatively assessed in the P, F_1,

and F_2 generations of the cross. Also, trichocyst discharge phenotypes were determined through the generations of the cross.

Although this type of cross has been reported previously (e.g. Grimes and Aufderheide 1991), quantative aspects of the size and cellular location of the inversion have not previously been reported.

CHAPTER II

EXPERIMENTAL PROCEDURES

Cell culture

Paramecium tetraurelia was cultured at 27˚C following established techniques of daily isolation of individual cultures and mass culture (Sonneborn 1950; Sonneborn 1970). A liquid medium, 0.15% baked lettuce extract augmented with stigmasterol, buffered with 5.25 mM sodium phosphate (pH 7.2), and inoculated with a nonpathogenic strain of *Klebsiella pneumoniae* (ATCC #27889), was used to cultivate the cells (Sonneborn 1970; Aufderheide *et al.*1999).

Parental cell lines

Wild-type trichocyst, inverted cortex line

The *P. tetraurelia* cell line with a region of inverted cortex, wild-type for the *nd6* allele, was a subisolate from the InvE line with a large cortical inversion, generated in 1980 (Aufderheide *et al.* 1999). The swimming behavior of cells with an inversion gives an indication of the extent of the inversion. As the number of rows inverted increases, the cells' swimming pattern looks markedly more "twisty." A cell line with a characteristically less "twisty" swimming pattern was selected to obtain a stable inversion size of approximately 4–5 inverted rows.

The extent and location of the inversion was determined by light microscopy of cells stained by the modified Fernández-Galiano silver staining techniques (Fernández-Galiano 1994). The cell line for this cross was confirmed to be of mating type "E" by testing against known mating type lines (Sonneborn 1970).

Trichocyst mutation, normal cortex line

A stock of the *nd6* mutant, with a normally oriented cortex, was isolated and determined to be mating type "E". This was crossed with a wild-type "O" line to produce a line of *nd6* mutants of mating type "O". The *nd6* mutants of mating "E" were conjugated with stock 51s "O" cells, wild-type for the trichocyst mutation, using the established protocols (Sonneborn 1970). Tight pairs were isolated from the conjugant reaction mixture. Upon separation the two exconjugant cells were isolated. Subsequently, autogamy was induced. Dippell's stain (Dippell 1956) was used to determine if nuclear reorganization occurred. Autogamous lines were grown to about 10 fissions and then tested for mating type. A 1% tannic acid solution was used to confirm the *nd6* trichocyst nondischarge phenotype in a viable "O" line (Pollack 1974; Aufderheide 1977).

Genetic cross

P generation

Cells with normal cortical organization, homozygous for the *nd6* trichocyst nondischarge allele (*nd6/nd6,* N, mating type "O"*)* and cells with wild-type trichocysts and inverted cortex (*nd6*$^{+}$*/nd6*$^{+}$*,* I, mating type "E"*)* were cultured separately, starved to induce mating reactivity and mixed, according to established techniques (Bession *et al.* 2010; Sonneborn 1970). The corticotypes of the parental lines were determined using a modified version of the Fernández-Galiano silver carbonate staining (Fernández-Galiano 1994). Five conjugate pairs were isolated from the mating reaction, and upon completion of conjugation, the cells of each pair were separated and separately cultivated as sister exconjugant F_1 lines.

F_1 generation

Successful sexual exchange between P generations was determined by scoring for the trichocyst phenotype in the F_1 generation; the F_1 cells were expected to be heterozygous for the marker allele and therefore would express the wild-type phenotype for trichocyst discharge (*nd6*$^{+}$*/nd6*). The corticotypes of the ten F_1 lines were determined using a modified version of the Fernández-Galiano silver carbonate staining (Fernández-Galiano 1994). The F_1 lines were cultivated through at least 20 divisions after conjugation, after which autogamy was induced to produce the F_2 generation.

F_2 generation

Dippell's staining for nuclear reorganization was used to determine if successful autogamy, self-fertilization that produces instant homozygosity at all loci, occurred in the F_1 generation to produce the F_2 offspring (Beale 1954**).** Five F_2 lines were isolated from each autogamous F_1 line. The corticotypes of the F_2 lines were determined. The trichocyst discharge phenotypes of the F_2 generation were determined.

Phenotype determinations

Fernández-Galiano staining

A modified version of the Fernández-Galiano silver carbonate staining technique was used to stain cells of the P, F_1, and F_2 generations to assess cortical status (Fernández-Galiano 1994).

Corticotype determination

Stained cells were examined using oil-immersion, brightfield optics. Twenty cells from each line were assessed for a total of 40 P, 200 F_1, and 1000 F_2 cells counted. The number of rows from the left side of the oral apparatus to the inversion, the number of inverted rows, and the total number of ciliary rows on the cell surface were counted for each cell selected. Rows were counted starting from the inner left side of the oral apparatus, around the body, to the right side of the oral apparatus to assess the relative location of the inversion through each generation. The corticotype ratio is a proportion

of the number of inverted rows per normal row of the cellular cortex.

Trichocyst scoring

Trichocyst discharge phenotypes were scored by mixing a drop of saturated picric acid solution or 1% tannic acid solution with a drop of the cell line to be scored (Pollack 1974). Cells from each F_1 and F_2 line were scored for trichocyst secretion. Dark field optics were used to determine trichocysts discharge or nondischarge during fixation. Trichocyst discharge indicated genotypes $nd6^+/nd6^+$ or $nd6^+/nd6$, while no discharge indicated genotype *nd6/nd6.*

Statistics

A chi-square test was used to compare the expected frequencies of the trichocyst phenotypes to the expected Mendelian ratios. An analysis of variance statistical test (ANOVA) was utilized to evaluate the changes in cortical status, namely number of inverted rows, from each generation. The mean and standard deviation from each generation was obtained.

CHAPTER III

RESULTS

General

Ciliary rows are composed of repeated cortical units; each cortical unit is organized around one or two basarl bodies. Each unit shows a distinct cytoskeletal and membraneous organization with an anteroposterior polarity (A-P) and a left-right asymmetry (L-R). Figure 1 shows a diagram of a cortial unit. Each cortical unit consists of one or two ciliary basal bodies and associated structures including a kinetodesmal fiber and a number of microtubular and membranous components. Figure 2 shows images of a normally oriented cell cortex.

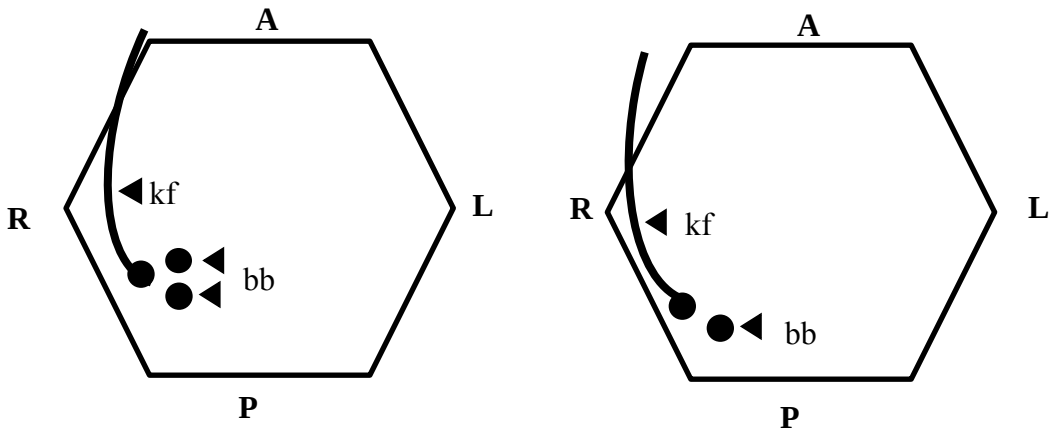

Figure 1 – Cortical units. Diagram of cortical units containing a kinetodesmal fiber (kf) and one or two basal bodies (bb). Note the distinct polarity and asymmetry of the cortical unit. Other cortical unit components are not shown for simplicity.

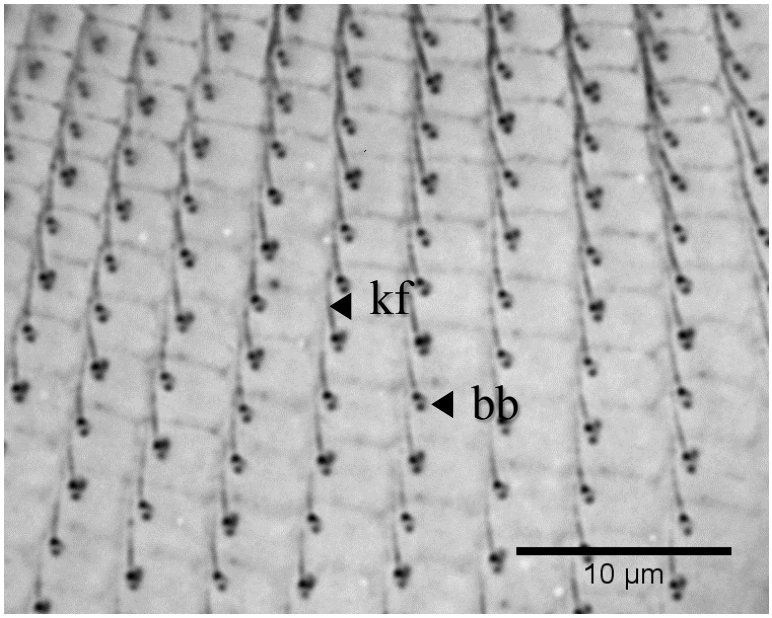

Figure 2 – Normal cell cortex. Normal cortical organization of *P. tetraurelia*, stained with the Fernández-Galiano silver carbonate protocol. Note the orientation of the cortical units and the direction of the kinetodesmal fibers. The anerior of the cell is to the top of the figure and the left side of the cell is to the right.

The interfaces between normal cortex and a region with inverted ciliary rows show two discontinuities: the wide juncture (wj) and end at the narrow juncture (nj), Figure 3 (Aufderheide *et al.* 1999). These junctures arise as the result of the spatial asymmetry of normal and inverted cortical units.

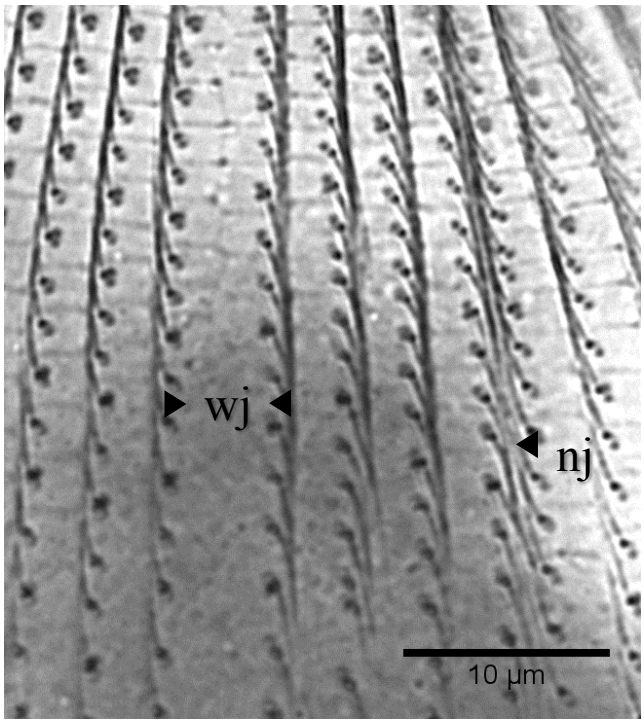

Figure 3 – Inverted cell cortex. Junctures between normally oriented cortex and inverted cortex in *P. tetraurelia* stained using a modified version of the Fernández-Galiano staining technique.

Trichocysts are secretory organelles that are distributed regularly along the surface of *Paramecium*. They are associated with the plasma membrane at insertion or docking sites between cortical units in the surface. When the cell is stimulated by exposure to picric acid or a 1% tannic acid solution, exocytosis of the trichocysts is triggered and they are explosively released from the cell in about 1 ms. The *nd6* mutation was used in this genetic study as a marker for Mendelian inheritance patterns. Figure 4 shows the wild-type trichocyst discharge response pattern to 1% tannic acid solution, while Figure 5 shows trichocyst nondischarge due to the *nd6* mutation.

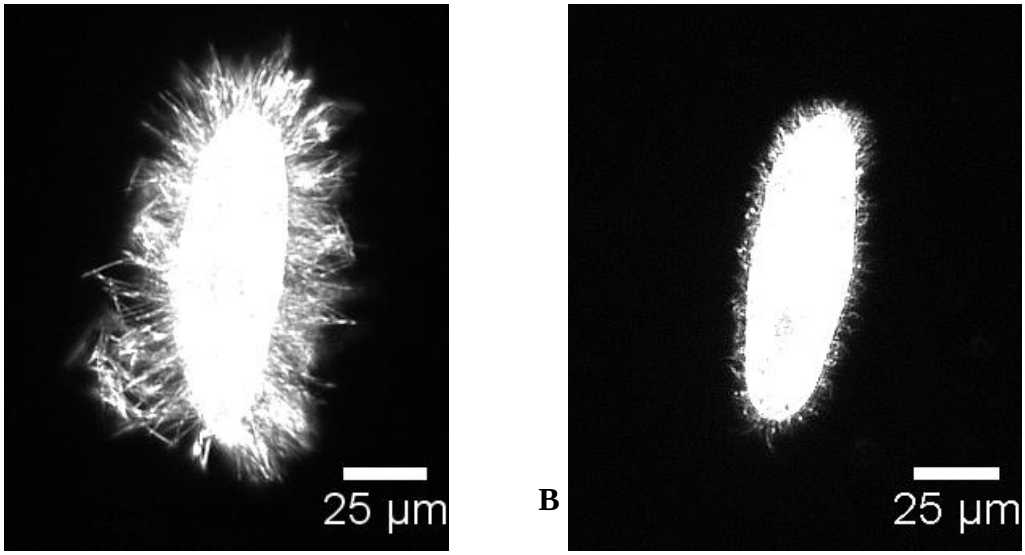

Figure 4 – Trichocyst phenotypes. (A) Wild-type trichocyst discharge. Note the spear-like array of discharged trichocyts. (B) Non-discharge phenotype of the mutant *nd6/nd6* phenotype. Cilia may be observed, but trichocysts are not discharged in response to the 1% tannic acid solution.

Parentals

A genetic cross was initated between two parental lines: *nd6*$^{+}$*/nd6*$^{+}$, with a cortical inversion (I), mating type "E", and *nd6/nd6*, normal cortex (N), mating type "O". Fernández-Galiano staining was used to determine the cortical pattern of the parental generation (Fernández-Galiano 1994). Table 1 displays the mean corticotype values of *nd6*$^{+}$*/nd6*$^{+}$ wild-type trichocyst, inverted cortex cells, and *nd6/nd6* trichocyst nondischarge, normal cortex cells, that were counted from mass cultures. The *nd6*$^{+}$*/nd6*$^{+}$, I, mating type "E" line had a mean of 76.25 rows of cilia and the inverted region was a mean of 4.55 rows and was an average of 27.85 rows from the oral apparatus. The *nd6/nd6*, N, mating type "O" line had a mean of 76.2 rows of cilia.

Table 1 Analysis of parental cortexes

	Parentals				
	nd6^{+}*/nd6*$^{+}$, I, mating type "E"			*nd6/nd6* N, mating type "O"	
	R	I	T	I	T
Mean	27.85	4.55	76.25	0	76.2
Standard Deviation	3.34	1.19	2.95	0	3.37
n	20	20	20	20	20

Parental corticotypes. R is the number of rows to the inversion from the inner left side of the oral apparatus, I is the number of rows inverted and T is total number of ciliary rows.

F_1 generation

Five exconjugatant pairs were successfully isolated from the reaction mixture. Twenty cells from each exconjugant cell line were counted for corticotype determination, producing a total of 100 cells with inverted cortex and 100 cells with normal cortex. Table 2 displays the cortical patterning of the F_1 generation. In the F_1 generation, only cells carrying the inversion before conjugation propagated the inversion pattern. The location of the inversion did not vary significantly from its position in the parental line. Cell lines that did not carry the cortical inversion before conjugation did not acquire an inversion after the cross. The number of total rows between parents and offspring did not change significantly.

Table 2 Analysis of F_1 cortexes

	F_1				
	Inverted Cortex			Normal Cortex	
	R	I	T	I	T
Mean	28.11	3.78	76.52	0	76.47
Standard Deviation	3.15	1.33	3.32	0	3.05
n	100	100	100	100	100

F_1 generation corticotypes. R is the number of rows to the inversion from the inner left side of the oral apparatus, I is the number of rows inverted and T is total number of rows.

F_2 generation

The F_2 generation was obtained inducing autogamy in the F_1 generation. Autogamy in paramecia produces homozygous genotypes in cells emerging from the process. Thus, a heterozygote population before autogamy will produce cells that are homozygous for one or the other allele in the heterozygous combination. The expected ratio of homozygous genotypes emerging from a heterozygote going through autogamy is 1:1 (Beale 1954). Table 3 shows the cortical patterning of the F_2 generation. Inverted ciliary rows were seen only in F_2 cells derived from F_1 cells that had an inversion before autogamy. The number of ciliary rows inverted in the F_2 line did not disappear or decrease drastically. In F_2 lines derived from cells with normal cortex in F_1, no inverted ciliary rows appeared. The number of total rows in the F_2 lines did not vary significantly with the F_1 generation.

Table 3 Analysis of F_2 cortex

	F_2				
	Inverted Cortex			Normal Cortex	
	R	I	T	I	T
Mean	26.67	3.16	76.68	0	76.19
Standard Deviation	4.37	1.17	2.60	0	2.94
n	500	500	500	500	500

F_2 generation corticotypes. R is the number of rows to the inversion from the inner left side of the oral apparatus, I is the number of rows inverted and T is total number of rows.

An analysis of variance (ANOVA) test was done to determine if any significant changes occurred in total number of rows, number of rows inverted, or the location of the inversion between the generations of the cross. Our results indicate that the number of total rows and the location of the inversions did not vary significantly, but the number of inverted rows seemed to slowly diminish, following a linear regression model with a loss of approximately 0.654 inverted rows per sexual reorganization. Refer to Figure 6 for a graphical representation of these results.

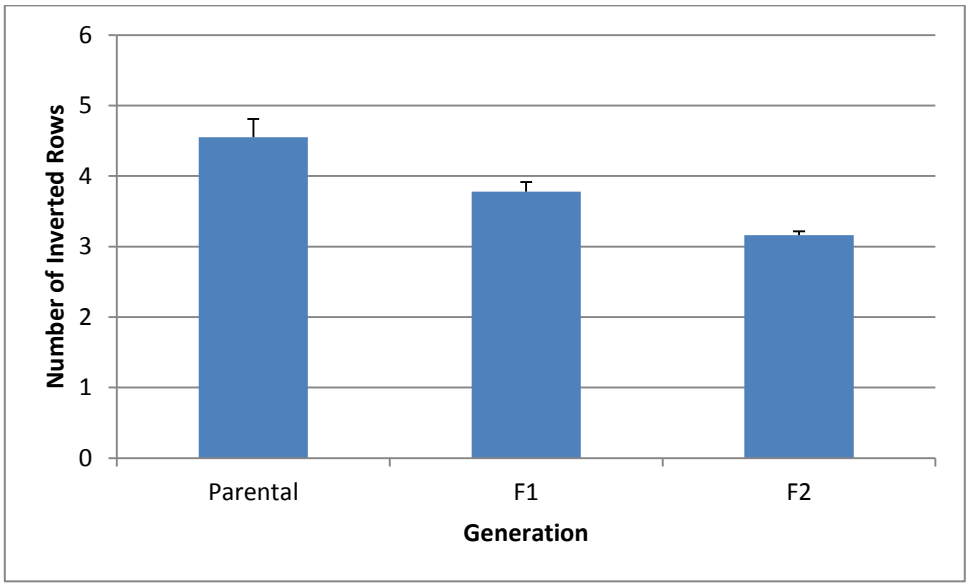

Figure 5 – Number of inverted rows over generations. Number of inverted rows present in each generation. Standard error bars displayed.

Trichocyst phenotypes

The *nd6* Mendelian marker gene was traced through each generation of the cross (Pollack 1974). Table 4 shows the inheritance of the *nd6* trichocyst allele through the genetic cross. In the parental generation, all cells with inverted cortex showed wild type trichocyst discharge, while the cells with normal cortex expressed the recessive, loss-of-function, *nd6/nd6* trichocyst mutation. The F_1 generation cells had a heterozygous genotype of $nd6^+/nd6$, as indicated by the wild-type trichocyst discharge phenotype of all F_1 cells. Of the 50 cell lines scored from the F_2 generation, 27 displayed wild-type trichocyst discharge patterns ($nd6^+/nd6^+$), while 23 of the cells display a nondischarge phenotype, indicating they were *nd6/nd6* genotype. This difference was determined to be not significant by the X^2-test.

Table 4 Trichocyst phenotypes

Generation	Genotype	Phenotype
	$nd6^+/nd6^+$	++
Parental	$nd6^-/nd6^-$	-
F_1	$nd6^+/nd6^-$	++ all (10)
	$nd6^+/nd6^+$	++ (27)
F_2	$nd6^-/nd6^-$	- (23)
	X^2=0.320	$P_{.05}$ = 0.572 N.S

CHAPTER IV

SUMMARY AND CONCLUSIONS

Our results indicate that the inheritance of the cortical inversion shows an epigenetic pattern, unlike the typical Mendelian pattern shown by nuclear loci. There seems to be no discernible Mendelian genetic component in the inheritance of inverted cortex through the standard F_1 and F_2 of a genetic cross.

In the F_1 generation, only cells carrying the inversion before conjugation propagated the inversion pattern to their progeny. Parents that did not carry the cortical inversion before conjugation did not acquire an inversion after the cross. Following autogamy, an abrupt loss of inverted ciliary rows in the F_2 lines containing an inversion, or the appearance of inversions in F_2 lines derived from cells with normal cortex, was not observed. Inverted ciliary rows were seen only in F_2 cells derived from F_1 cells that had an inversion before autogamy. Had the inversion followed a Mendelian pattern of inheritance, as demonstrated by the *nd6*$^+$ allele, we should have seen the inversion appear or disappear in the F_1 and F_2 generations of both exconjugant sister cell lines.

The total number of ciliary rows did not vary significantly through each generation. Additionally, the location of the inverted rows in the circumference of the cell, as measured by the number of rows from the left side of the oral apparatus, did not vary significantly in position among the P, F_1 and F_2 lines. However, statistical analysis of the cells carrying the inversion indicated that the size of the inversion pattern showed a decrease at a significant rate of approximately 0.65 rows per generation. An explanation for this event may be that the inversion is inherently unstable during vegetative proliferation, and may decay or disappear when it is not actively selected for. Further research would be required to accurately assess this phenomenon. Regardless of its implications, this observed slow loss of inverted rows through each generation does not negate the phenomenon of cortical inheritance: only the linear, structural descendants of cells originally carrying inversions expressed inversions.

Our hypothesis was confirmed: cortical inversions are inherited only through the structural descendants of a cell originally expressing an inversion. The heritability of the *nd6* gene is consistent with a typical Mendelian pattern, but heritability of the cortical inversion does not show a Mendelian pattern in the F_1 or F_2 generations.

Though nuclear genes that code for the proteins of the cortex are obviously necessary to perform morphogenesis, some aspectes of the organization of the cortex appear to be inherited in a non-Mendelian fashion. This phenomenon, described since the 1960s (Beisson and Sonneborn 1965), suggests that the complex three-dimensional architecture of the cell can influence how and where new protein components are assembled and patterned, and therefore express a heritability distinct from that of nuclear genes or even cytoplasmic genes. This form of inheritance of structural organization has been variously termed cytotaxis (Sonneborn 1962), or directed assembly (Grimes and Aufderheide 1991), and reminds us that the fundamental reproductive unit of life is the cell, within which the molecular machinery of transcription and translation operates.

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