STRUCTURAL INHERITANCE AND SWIMMING PATTERN IN *Paramecium tetraurelia* CELLS WITH CORTICAL INVERSIONS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Structural Inheritance and Swimming Pattern in *Paramecium tetraurelia* Cells with Cortical Inversions. (May 2014)

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Paramecium tetraurelia cells with cortical inversions are characterized by a 180° rotation of all the components of the ciliary rows (basal bodies and their ancillary structures) in a portion of the cortex. The power stroke of inverted cilia is “wrong-way” (towards the anterior) and produces a distinctive twisty or rotary swimming pattern. Although cortical inversions are heritable, some degree of artificial selection has been necessary to maintain the twisty swimming phenotype. The purpose of this project was to determine whether changes in the composition of the cortex are directly responsible for the “better” swimmers, as opposed to the cells adapting their swimming behavior by some other means. Another goal was to develop a quantitative relationship between swimming pattern (velocity, helical width and pitch) and number of inverted ciliary rows. We have found that cell populations can in fact spontaneously lose (or gain) inverted rows, and that, intuitively, inverts with fewer inverted rows are faster, swim straighter, and travel greater distances per helical turn. This suggests variants with fewer rows inverted have a functional and energetic advantage in competing for food resources in mass culture, resulting in an observed decline of mean inversion size over time. Overall, it is apparent that the cortex can undergo dynamic changes with measurable effects on swimming phenotype. These changes, divorced from any sort of genomic changes, nonetheless have profound effects on both cortical and

behavioral phenotype, and can be stabilized or destroyed depending on the prevailing selective forces. This suggests structural inheritance may be highly significant to cortical stability and evolution in *Paramecium.*

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I am entirely indebted to the guidance and assistance of Dr. Karl Aufderheide, who has given me the opportunity to create something for myself and explore a beautiful model organism.

I am also exceedingly thankful for the moral and technical support of Shanda Griffin, whom I'm sure I'll marry as soon as I gather a few things – maybe a ring and my diploma.

CHAPTER I

INTRODUCTION

The genus *Paramecium* contains free-living, heterotrophic protists that are members of Phylum Ciliophora (ciliated protozoa). Although unicellular, paramecia are remarkably complex, possessing a number of specialized organelles for feeding, osmoregulation, and waste excretion (Beale 1954).

Like all ciliates, paramecia possess a highly sophisticated array of surface-related fibrous and membranous structures known as the cortex (Aufderheide, et. al., 1980). These structures are organized into thousands of repeated monomers called cortical units. Each unit is organized around one or two basal bodies and contains specialized sets of microtubules, alveolar membrane differentiations, and other cytoskeletal components in a precise and consistent arrangement that grants each unit a definite anteroposterior (AP) polarity and left-right (LR) asymmetry (fig. 1). Cortical units run longitudinally in parallel rows known as kineties or ciliary rows which cover the entire surface of the cell (Aufderheide and Grimes 1999). Within a ciliary row, the individual cortical units share the same directionality (fig. 2), granting each row definite AP and LR axes.

Figure 1: Schematic representation of a cortical unit.

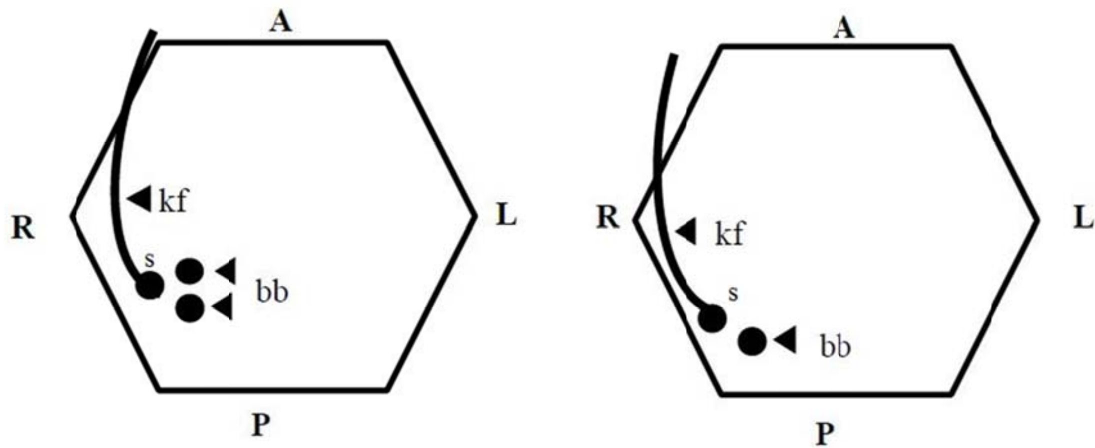

Fig. 1: Diagram of a cortical unit, illustrating visible ancillary and cytoskeletal and membranous structures organized about one or two ciliary basal bodies [bb], as they appear when stained via the Fernandez-Galiano technique. (Fernandez-Galiano 1976, 1994). A striated protein band known as the kinetodesmal fiber [kf] (which actually emanates from the basal body itself) appears to taper off to the anterior right from the parasomal sac [s]. Each cortical unit has a definite anteroposterior (AP) polarity and left-right (LR) asymmetry. (Made by Rebecca Cross).

Figure 2: Wild-type cortex.

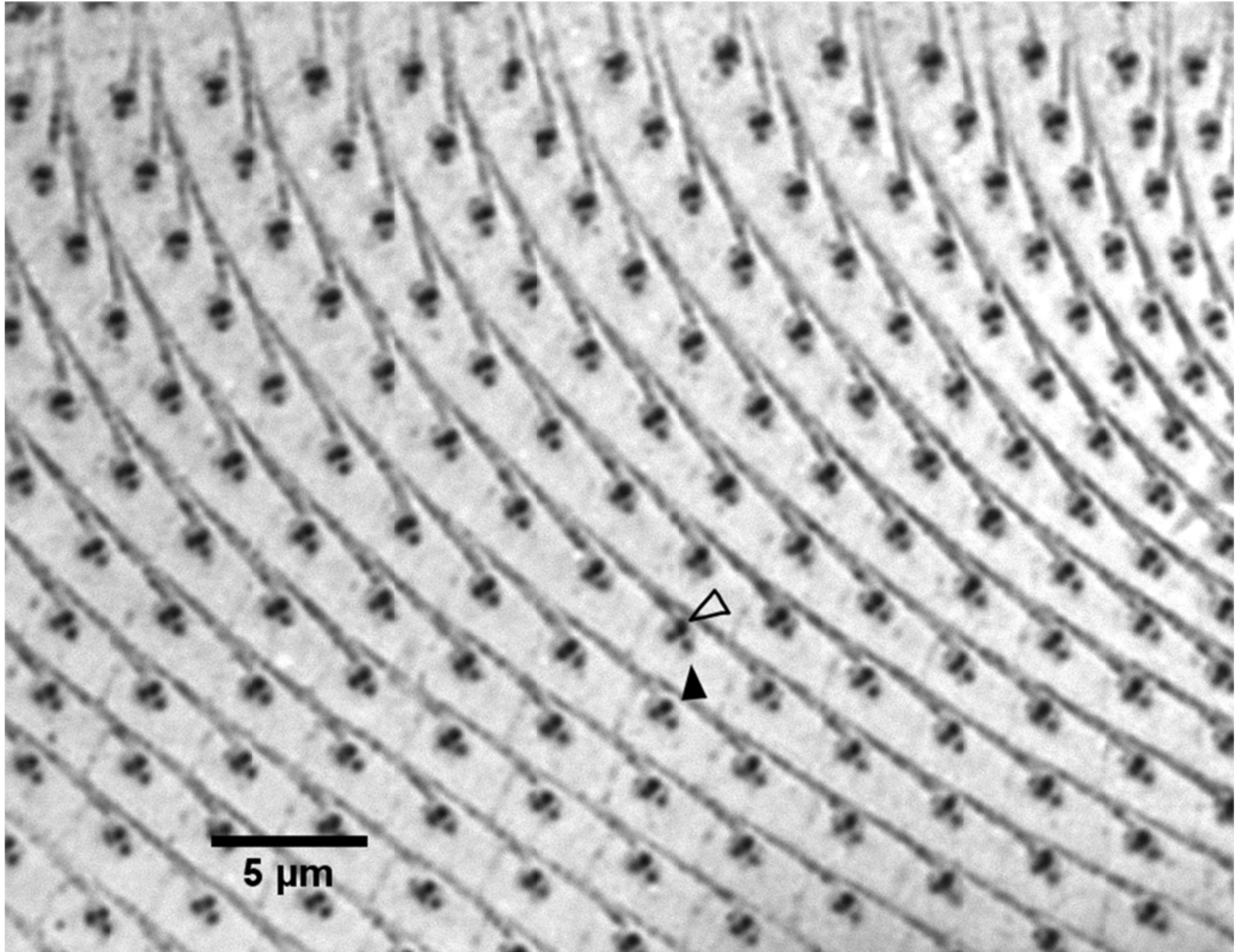

Fig. 2: Fernandez-Galiano silver carbonate stained cortex of *P. tetraurelia.* Black arrowhead: a ciliary basal body; white arrowhead: parasomal sac. The kinetodesmal fibers, overlapping and directed towarded the anterior, are also preferentially stained, while the alveolar membranes are faintly visible.

During each cell cycle (roughly every 4.5 to 5 hours at 27° C, given ample food), the number of cortical units must be duplicated to ensure each daughter cell is granted a full complement of cortical structures. Somatic morphogenesis begins with the nucleation of new basal bodies perpendicular and to the immediate anterior of existing basal bodies, followed by the assembly

of the ancillary structures around the new basal body and its eventual sequestration from the "parent" cortical unit (Dippell 1968; Beisson 2008; Aufderheide et al. 1980; Grimes and Aufderheide 1991). Basal body proliferation appears to be coordinated with the onset of oral morphogenesis, and is concentrated about the middle of the cell; basal bodies near the future division furrow nucleate anywhere from one to four daughters, while those closer to the poles might nucleate a few daughters, if any (Beisson 2008; Aufderheide et al. 1980).

New basal bodies apparently require the parental body as a nucleating site for proper assembly and positioning at the cell surface; paramecia are unable to assemble new somatic cortical units *de novo* (Beisson 2008, Aufderheide et al. 1980). An existing basal body presumably provides the local environment, mediated by direct links or scaffolding elements, necessary for the correct assembly and orientation of new basal bodies and their associated ancillary structures; this subsequently includes replicating the geometry of the parental cortical unit and preserving the asymmetry and polarity of the entire ciliary row (Iftode and Fleury-Aubusson 2003). Thus local patterning in the cortex is based on a sort of templating mechanism known as cytotaxis or directed assembly (Sonneborn 1964, Sonneborn 1970a, Aufderheide et al. 1980), wherein the positioning and polarity of new cortical units is determined by the organization of preexisting cortical units.

At the level of gene expression, we see that the macronucleus exerts its influence on cell surface patterning through 1) the genes coding for the primary amino acid sequences of individual cortical unit components, and 2) regulatory control of the cell cycle and related events, including the spatial and temporal aspects of morphogenesis (Sperling 1991). At a posttranslational level,

however, the arrangement of preexisting surface structures provide a major source of information used to regulate the assembly of new cortical units (Beisson and Sonneborn 1965; Sonneborn 1975; Aufderheide 1980; Aufderheide and Grimes 1999). Thus the inheritance of cortical structures is not sufficiently explained by Mendelianism or a simple self-assembly model for the cortical proteins (Aufderheide 1991). We will also see that directed assembly permits the transmission of abnormal cortical arrangements that are *not* the result of heritable genetic mutation, as demonstrated by *P. tetraurelia* cells with cortical inversions.

Cells with cortical inversions are characterized by a 180° rotation of a portion of the cortex. All components of the ciliary rows in this region (basal bodies and their ancillary structures) are rotated 180° in the local plane of the cortex — the inversion does not disturb the internal organization of individual cortical units (Aufderheide and Grimes 1999). The rotation does however change functional aspects of the locomotory structures: the power stroke of the inverted cilia is directed to the cell's anterior (rather than the normal backwards stroke) and produces a distinctive twisty or rotary swimming pattern, easily discernible with a dissecting microscope (Aufderheide, et. al., 1980; Aufderheide and Grimes 1999).

Since the propagation of individual basal bodies in a ciliary row requires preexisting structures within the ciliary row as regulators of positioning and assembly, inverted units replicate themselves in their "reverse" orientation and the abnormal region is inherited by new clones (Aufderheide and Grimes 1999). It has been explicitly shown that replacing the genome of inverted lines does not destroy inheritance of the inverted region and that, conversely, genetic crosses of inverted lines with "wild types" does not grant the normal cells an inversion (Beisson

and Sonneborn 1965; Sonneborn 1970a; Cross unpublished data). Hence the inverted phenotype, although heritable, is not a consequence of a genetic mutation or a particular genotype, and is transmitted only to structural descendants of the inverts.

While cortical inversions are transmitted through asexual divisions, and are not destroyed by genomic reorganizations during conjugation, some degree of artificial selection based on the twisty swimming phenotype has been necessary to maintain cell lines with cortical inversions (Aufderheide 1999). The extent or size of the inversion, in total number of inverted rows, is presumably directly related to swimming pattern (Tamm, 1972). Occasionally, in a culture of extremely twisty cells, variants will arise with patently straighter swimming, but still some degree of "relaxed" twisting or wobbling as they move forward. It has been of great interest as to how these variants are produced, and whether they truly represent spontaneous rearrangements of the cortex resulting in the loss of inverted ciliary rows.

Experimental objectives and hypotheses

One objective of this work has been to document morphogenesis in the inverted region, where the principle of directed assembly is readily apparent. We have also attempted to quantitatively follow potential changes in the composition of the cortex in the absence of specific selection for twisty swimming behavior. Intuitively, we hypothesize that the number of inverted rows will decrease over time due to competitive pressures (with a corresponding improvement in swimming ability); as cells lose inverted rows, they should gain an obvious selective advantage with respect to maneuverability and food-gathering ability. Alternatively, it is possible that the swimming variants are adapting their swimming behavior without alterations to the cortical

structures, somehow negating or reversing the "wrong-way" beat of some of the inverted cilia (i.e., they are rare behavioral mutants). While it may seem obvious to conclude the cells are simply losing inverted rows, this would require spontaneous, non-mutational rearrangements of the cell cortex that are far from what would be considered normal, and thus warrant documentation. A quantitative demonstration of the relationship, if any, between swimming pattern and the size of the inversion was also a major experimental focus.

Since variability is the prerequisite of biological change, structural traits and their inheritance can be likened to genes in this context, and analyzed from a population genetics perspective – though we must keep in mind that it is not nucleotide sequences that are changing, but spatial information related to the orientation of many molecular components, which in turn influences cell behavior.

CHAPTER II

MATERIALS AND METHODS

Culturing

Both the wild type cells and cells with cortical inversions used in this study are derived from *Paramecium tetraurelia* stock 51s, mating type O. Cells were cultured in a 0.15% baked lettuce powder infused medium (BL) buffered with 5.25 mM sodium phosphate (pH 7.2) and augmented with 5 mg/L stigmasterol. The day before use, the sterile media were inoculated with a non-pathogenic strain of *Klebsiella pneumonia* (ATCC #27889), to serve as a monoxenic food source. Cells were maintained at 27°C in siliconized 3-hole Pyrex spot plates and periodically reisolated according to established techniques (Sonneborn 1950, 1970b). Cells carrying cortical inversions - known as line InvE (TAMU:0380:12) - were maintained by periodic reisolation of cells with the "twisty" swimming phenotype. This line is characterized by some 12-15 inverted rows on the left dorsal side of the cell.

Microscopy and staining

Light microscopy was performed using an Olympus model BH-2 microscope. Living cells were imaged using a Nomarski differential interference contrast optical system on the same microscope. Typically living cells were first immobilized via rotocompression (Aufderheide 2008); the Aufderheide model and the Janetopoulos "Commodore" model microcompressors were used in this study (reference Cole, others –see Dr. Auf). Photomicrographs were taken using a Lumenera Scientific Infinity-2 camera with Infinity Capture software, and images were processed and labelled using ImageJ. Staining protocols used include the Fernandez-Galiano

ammoniacal silver carbonate method and Chatton-Lwoff silver impregnation method (Fernanded-Galiano 1976, 1994; Foissner 1991).

Corticotyping

The corticotype method is a means of characterizing a cell with cortical inversions. It is simply a standard and systematic way of counting the ciliary rows in a cell, and is used to determine three parameters: the "longitude" of the inversion, the number of rows inverted, and the total number of ciliary rows/kineties. The longitude is here defined as the number of normally-oriented rows counted from the left side of the oral apparatus until the first inverted row (the first wide juncture) is reached (the convention is to use the cell's left, as if one were looking out from the oral apparatus – see figure 3). We find that the counts are simplified by starting from the lip of the buccal overture/oral opening, since depending on the orientation of the cell under observation it is often difficult to accurately count rows as they continue down into the buccal cavity. For all the corticotype data obtained, counts begin and end at rows that completely or very nearly encircle the oral opening (begin *outside* the overture).

Figure 3: Schematic representation of the conventions for describing cell axes in *Paramecium*.

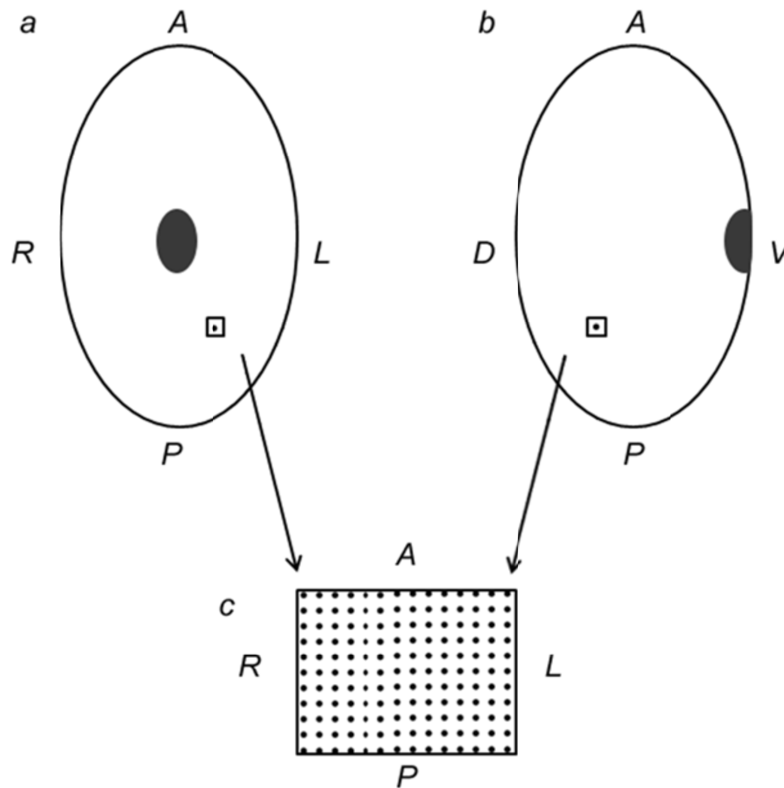

Fig. 3: a) View of a whole cell from the ventral side and b) the right side. c) shows a local patch of cortex with linear rows of cilia or basal bodies. The dark oval represents the oral apparatus, defining the ventral face of the cell. The global axes are defined with respect to the oral apparatus and the direction of cell movement. Local axes are defined from the viewpoint of within the cell, looking out. (Reproduced from *Cellular Aspects of Pattern Formation*, Grimes and Aufderheide 1991).

Investigating cortical instability and loss of inversion

To follow changes in the cortex in the absence of "artificial selection" with respect to swimming pattern, 10 cells from InvE were introduced to 5 mL of the BL media and allowed to proliferate for 24 hours at 27 degrees Celsius. Daily transfers of 1 mL of the previous day's mass culture into 4 mL of fresh BL media were then carried out for 5 consecutive days. Simultaneously, 2 mL

of cells from each daily mass culture were stained using the Fernandez-Galiano protocol; a sample of 20 silver-impregnated cells was corticotyped each day. This procedure was also carried out with an established subline of InvE demonstrating extreme twisty swimming (see fig. 6, Results).

Investigating swimming pattern and corresponding corticotype

A single cell demonstrating extreme rotary swimming was placed in about 6 mL BL media and allowed to proliferate for 72 hours at 27 °C. From this mass culture, a diverse array of swimming phenotypes presented itself. Individual cells were selected to establish sublines (labelled InvI-VI) of differential swimming pattern. These sublines were stabilized by selective reisolation of "representative" swimmers for approximately one week.

The swimming ability of each subline (and of wild type cells) was assessed quantitatively by measuring "trace images" of swimming paths. With darkfield optics and a sufficiently long exposure time, at low power magnification, images reveal the path taken by the cell as a bright streak, headed by the cell as it swims. Wild type cells, and inverts that can successfully swim laterally, produce a left-handed, approximately circular helix as they twist forward (Fig. 4). Coupled with an appropriate calibration image, properties of the cell's swimming path can be directly measured with software such as ImageJ. Velocity, helical diameter, and helical pitch were the parameters of interest. A two-second exposure was used for all images collected and measured for this study. Cells were cleaned with 2-3 washes in Dryl's buffer before being placed in a self-constructed "motility chamber" (a slide with a simple 6x6x1 mm enclosure made from rectangular pieces of cut slides) filled with Dryl's buffer.

Figure 4: Swimming trace image of a moderate invert.

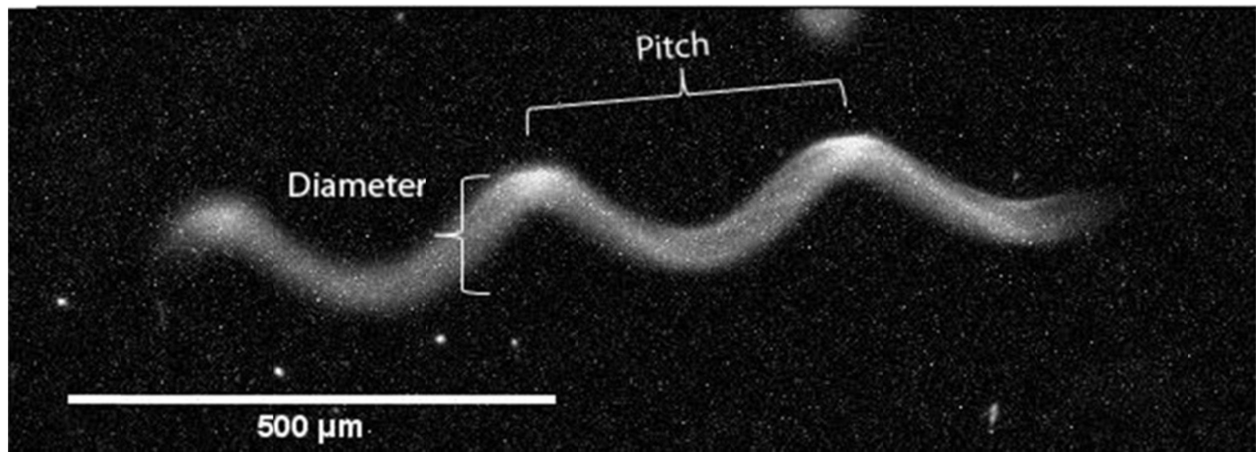

Fig. 4: Swimming trace image of a moderate invert (darkfield, 2-second exposure). Aspects of the swimming pattern can be accurately measured from these trace images, which outline the cell's helical swimming path. The path resembles a sinusoidal projection when cells swim laterally. Note that measurements of helical diameter presented in the Results section include the cell width, and measurements of displacement (velocity) include the cell length.

CHAPTER III

RESULTS

Loss of the inversion without selective reisolation

Figure 5: Mean inversion size, longitude, and total ciliary rows in the absence of selective reisolation with respect to swimming pattern in line InvE.

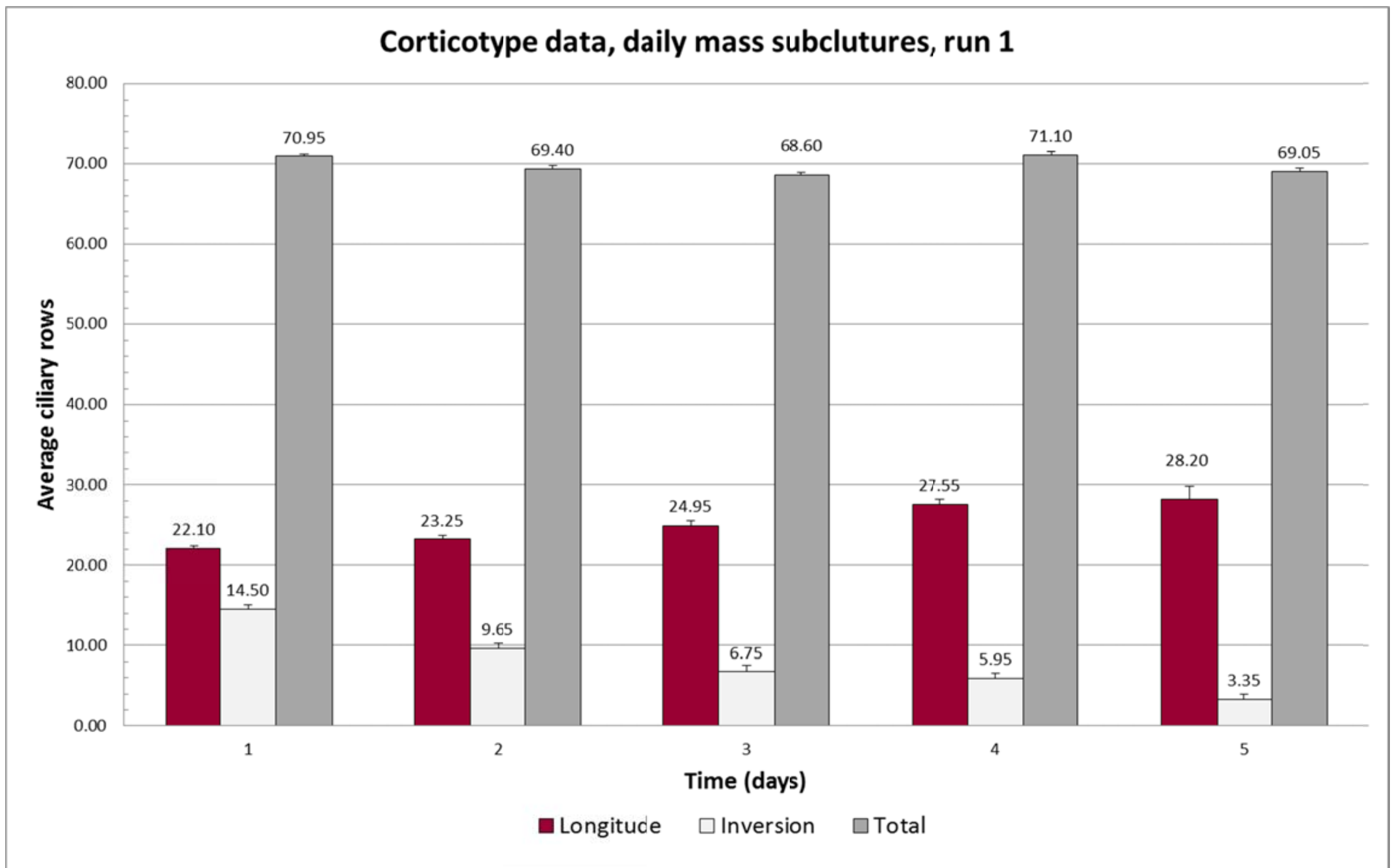

Fig. 5: A graph of the average number ciliary rows from the left side of the OA (longitude), average number of inverted rows, and average number of total rows outside the OA is shown. Standard error bars are included. Approximately every 24 hours 2 mL of cells were taken from

daily mass subcultures and stained with the Fernandez-Galiano method. A sample (n) of 20 silver-impregnated cells was then corticotyped and daily averages calculated. Handling of mass subcultures is described in the Materials and Methods section.

Figure 6: Mean inversion size, longitude, and total ciliary rows in the absence of selective reisolation with respect to swimming pattern in a subline of InvE with extreme twisty swimming.

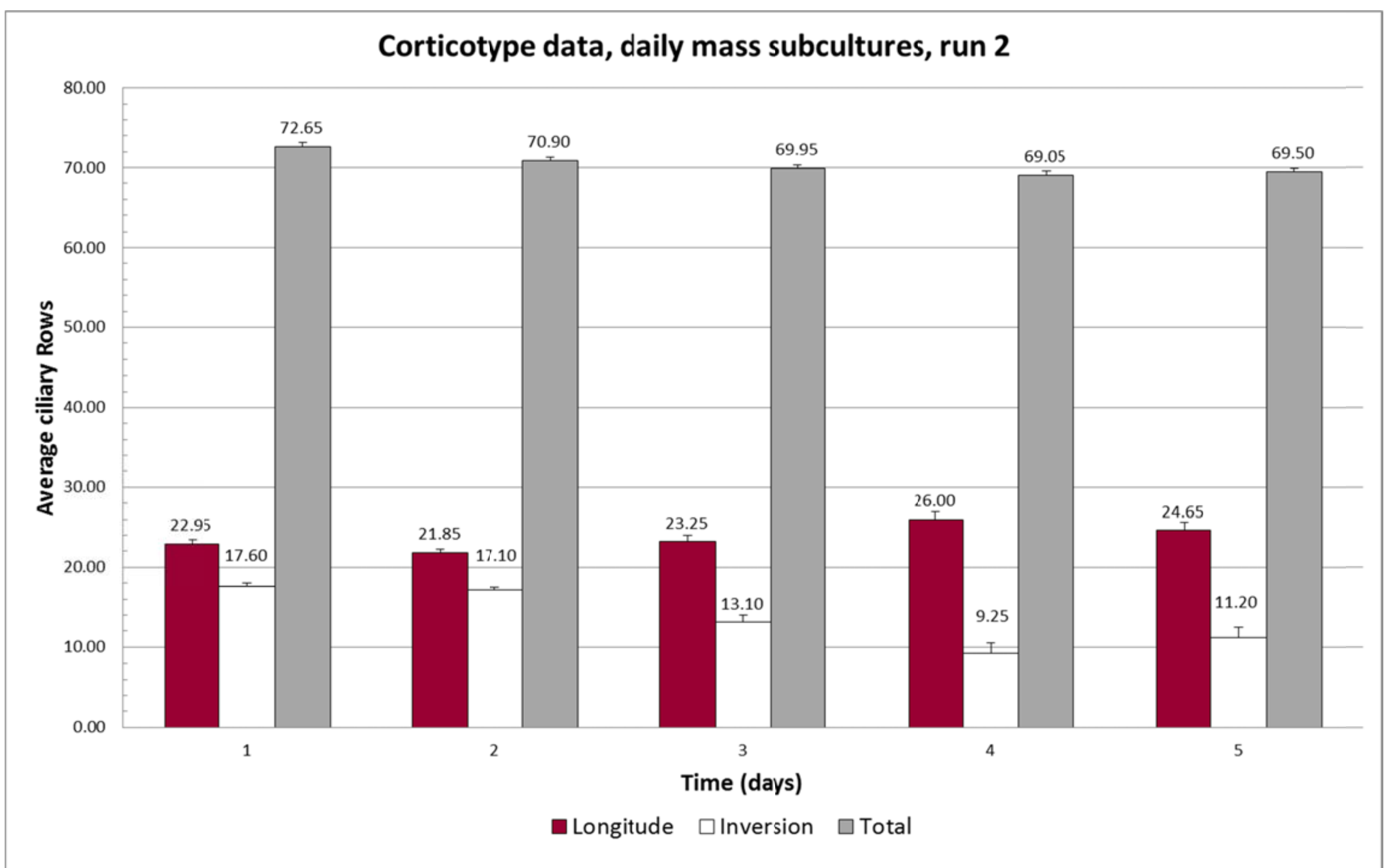

Fig. 6: A graph of the average number ciliary rows from the left side of the OA (longitude), average number of inverted rows, and average number of total rows outside the OA is shown, as in Fig. 5.

Examining figure 5, it is clear that the mean inversion size decreases over time without selective reisolation of twisty swimmers. We also see that the total number of somatic ciliary rows experiences a brief dip as inverted rows are lost, but it is neither as drastic nor durable a change as might be expected. Importantly, we also see that longitude increases as the mean inversion size decreases. Unless all of the inverted rows are somehow being converted into normally-oriented rows, this strongly suggests the cell is establishing new (normally oriented) rows in the cortex, presumably originating in the buccal cavity as suggested by others (Iftode and Adoutte 1991). *P. tetraurelia* may be able to maintain the total number of ciliary rows within a certain range, but it does not appear to be capable of differentiating inverted rows or cortical units from normally-oriented ones.

However, although a mechanism for establishing new somatic rows probably exists, it does not seem to be a continual process as suggested by the cortical slippage/rotation hypothesis (Iftode and Adoutte 1991), despite the fact cortical slippage is known to occur in the ciliate *Tetrahymena* (Nanney 1972). If new rows were continually established in the buccal cavity and circumferentially travelled about the cell as old rows terminated into the oral apparatus, the line of inverts used in this study (InvE - TAMU:0380:12) would be completely unstable and the inversion inevitably destroyed long ago, despite any effort to select for the trait indirectly based on the twisty swimming pattern. This process of morphogenetic regulation is probably induced only when the number of ciliary rows, sensed or approximated by the cell via unknown means, drops below a certain threshold. There has been no evidence of the inversion changing its location relative to the global axes of the cell as the cortical rotation process would require it to

do. Since its isolation in 1980, InvE has consistently shown an inversion (usually 12-15 rows in magnitude, with periodic selection as described previously) on the left dorsal side of the cell.

Furthermore, data in both figures convey periods of sudden, severe reduction in the average number of inverted rows within 24 hours (loss of nearly 5 inverted rows on average in fig. 5 from day 1 to 2; loss of 8 rows on average in fig. 6 from day 3 to 5). Although the trend is not absolute (we see slight increase in average number of inverted rows from day 4 to 5 in fig. 6), it appears that cells with large inversions are at a selective disadvantage in mass culture. As expected, the subline examined in fig. 6 has a greater average number of inverted rows (on day 1), corresponding to its more extreme swimming phenotype.

Lastly, the most obvious but highly significant interpretation to be gathered from these data is that spontaneous changes in the structural organization of the cortex are possible, indicating possible mechanisms (apart from genetic mutation, in light of earlier work) that can generate variability with respect to this trait in the population. Variants with fewer inverted rows appear to quickly out-proliferate cells with larger inversions in mass culture and can rapidly depress the mean number of inverted rows. Artificial selection has maintained (or increased, in the case of the extreme twisty subline examined in fig. 6) the size of the inversion; natural selection favors spontaneous variants with fewer rows inverted.

Microscopic observations of cortical instability within the inverted cortex

Figure 7: Morphogenesis and directed assembly.

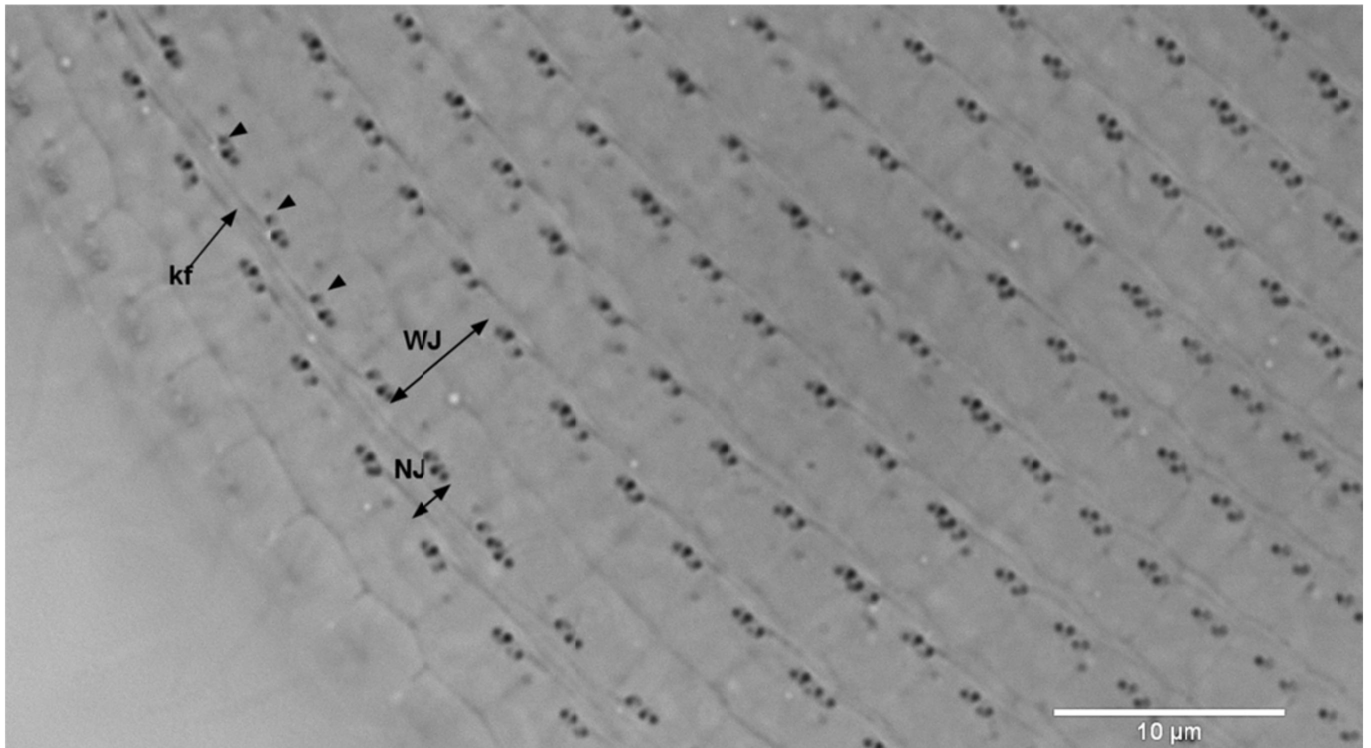

Fig. 7: Morphogenesis of new basal bodies (arrow heads) in an inverted ciliary row, visualized with the Fernandez-Galiano silver-carbonate stain. New basal bodies arise exclusively to the *local* anterior of parental basal bodies; in the inverted units, this means new units are directed to cell's posterior, preserving the abnormal polarity and asymmetry of the inverted ciliary row. WJ and NJ refer to the distinctive "wide" and "narrow" junctures invariably formed at the boundaries of inverted segments of the cortex.

Morphogenetic errors and disruption of global patterning in cells with cortical inversions.

Figure 8: Disruption of global patterning in the anterior pole of cells with cortical inversions.

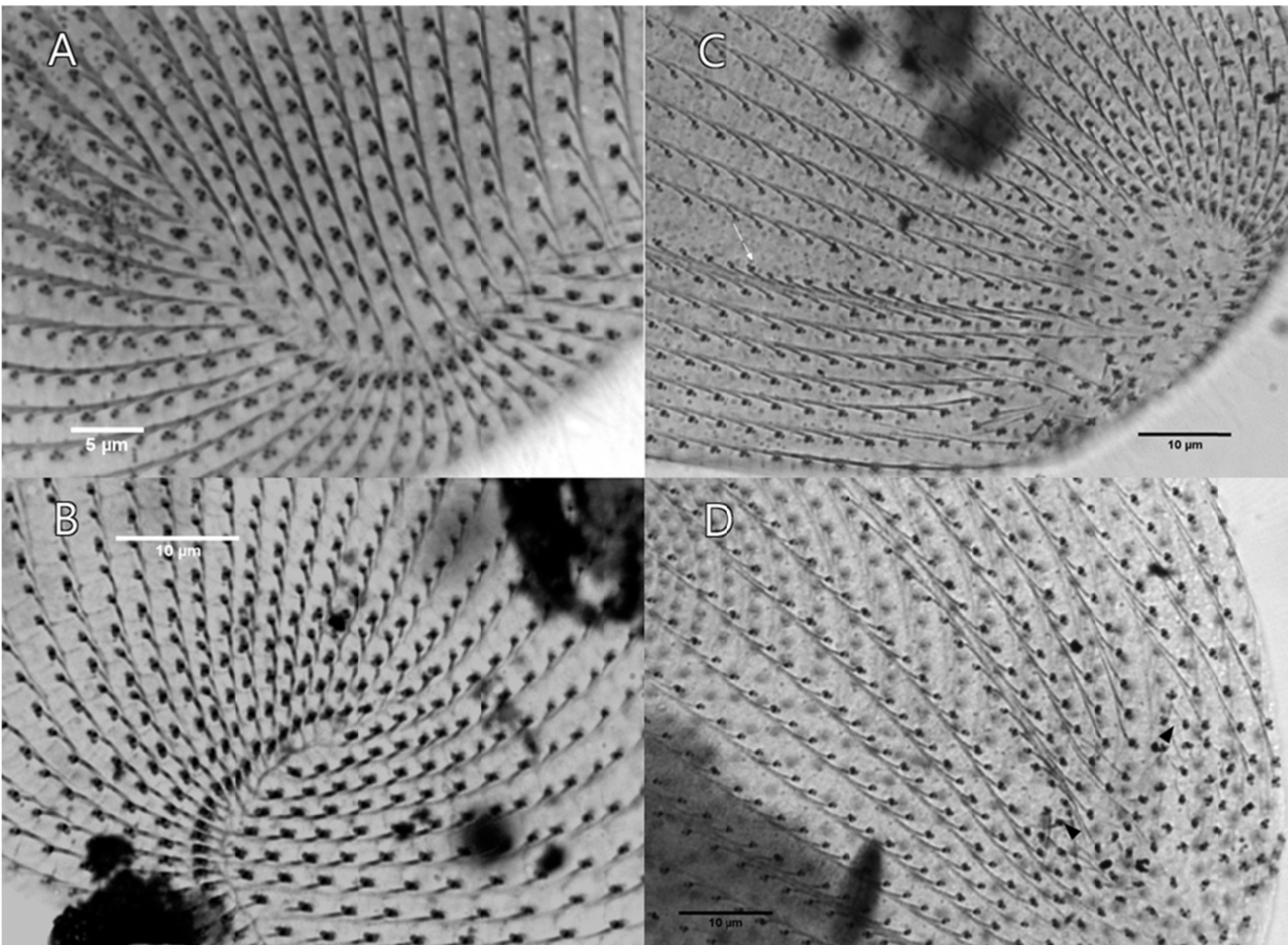

Fig. 8: A) and B) show the anterior pole of "wild type" cells possessing only normally-oriented ciliary rows. We see that the ciliary rows terminate neatly in a semi-circular arrangement. C) and D) show the anterior pole of cells with a large number of inverted ciliary rows. Note the chaotic and disordered placement of cortical units in this region. C) also shows an intervening normal row (white arrow) separating two inverted "domains". In D), the arrowheads indicate where rows that have failed to terminate in the polar region appear to rotate back into the dorsal cortex in the reverse orientation.

Figure 9: Ciliary row distortion near the cell midline preceding cytokinesis.

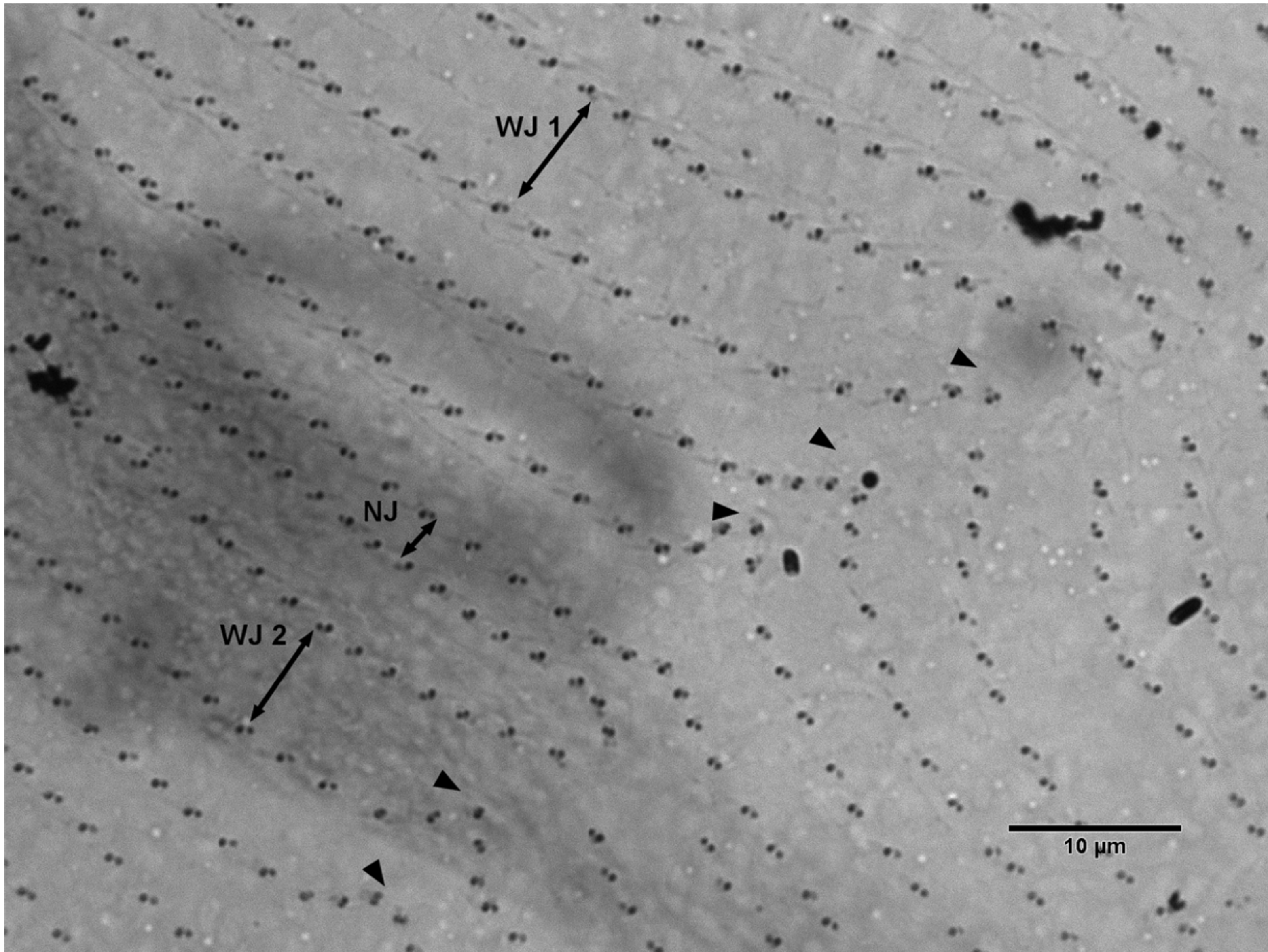

Fig. 9: Morphogenetic cortex surrounding the inverted rows near the future division furrow frequently appears to be stressed, with inverted rows heavily bent and distorted as seen here (arrowheads). Adjacent normal rows are also distorted into a sigmoidal shape.

Figure 10: Broken inverted rows.

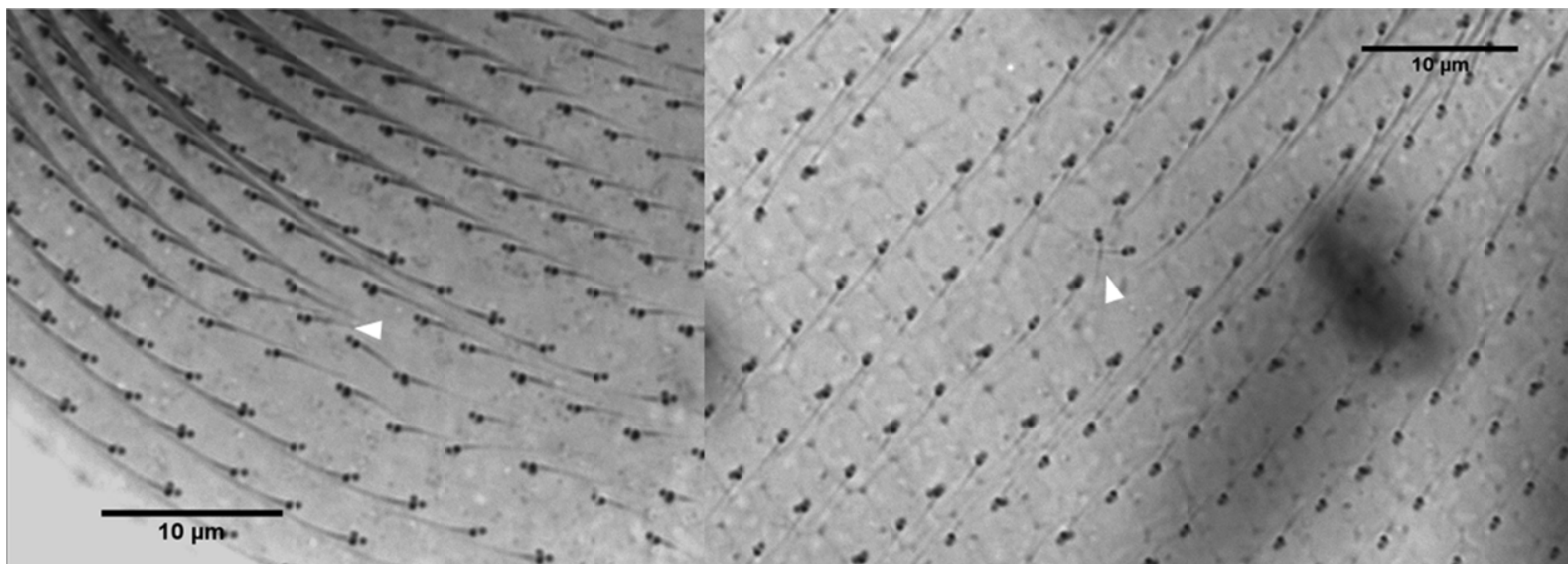

Fig. 10: For unknown reasons, it is common to observe short or "broken" inverted rows in cells with more than a small region (1 or 2 rows) of inverted cortex (arrowheads). These rows are probably not transmitted past the division furrow at cell division, which would result in the posterior division product losing the row and a measurable change in corticotype in cell progeny.

Cortical Inversions and Swimming Pattern

Figure 11: Swimming parameters and number of inverted rows.

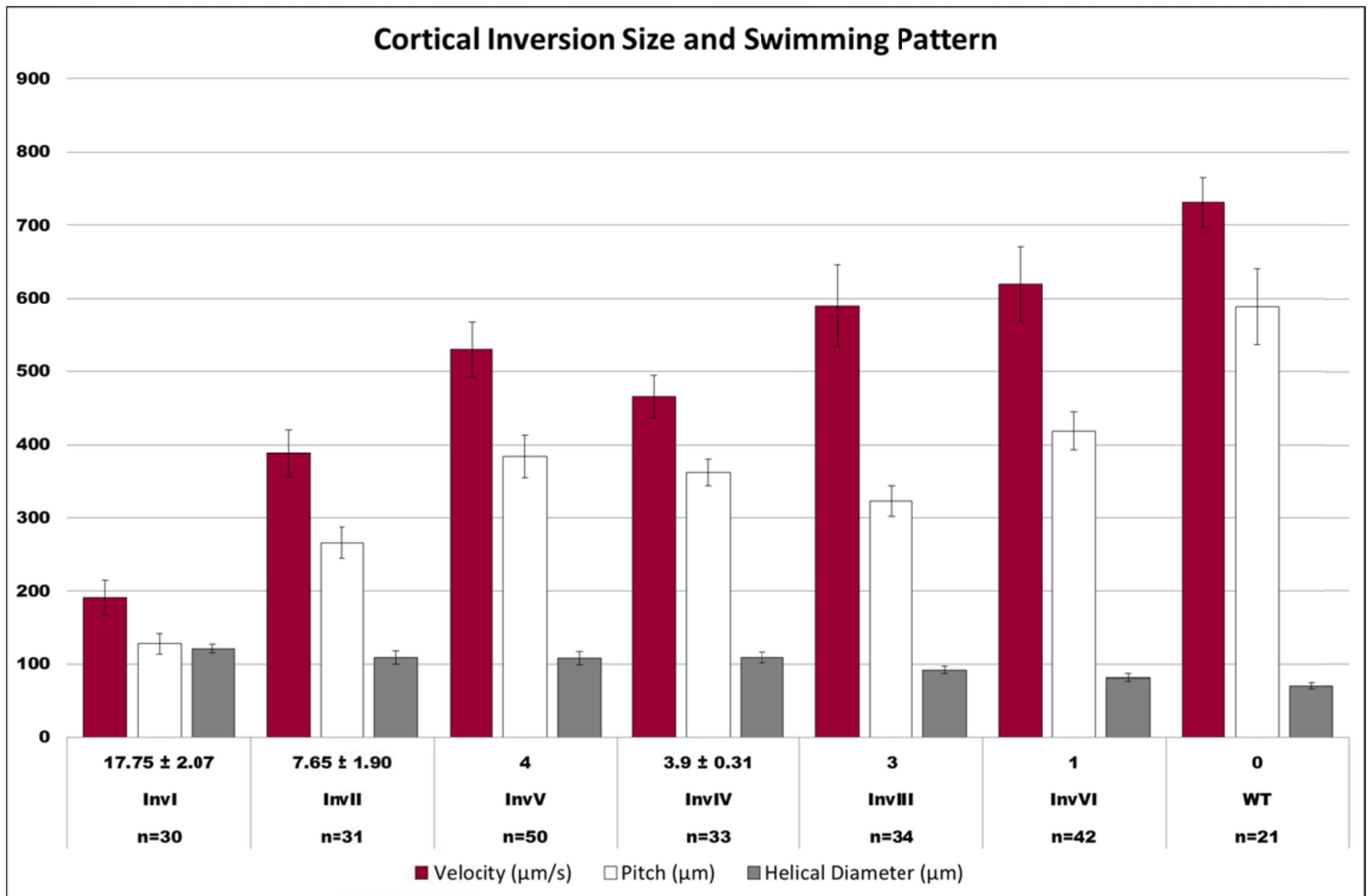

Fig. 11: The mean velocity, pitch, and diameter for samples of (n) cell swimming paths for sublines InvI-VI and wild type (WT) cells. Standard deviation bars included. The mean number of inverted rows ± SD for a sample of 20 cells from each subline (and WT cells) is shown along the horizontal axis; vertical axis units are shown in the figure legend.

Fig. 12: Swimming phenotype transitions.

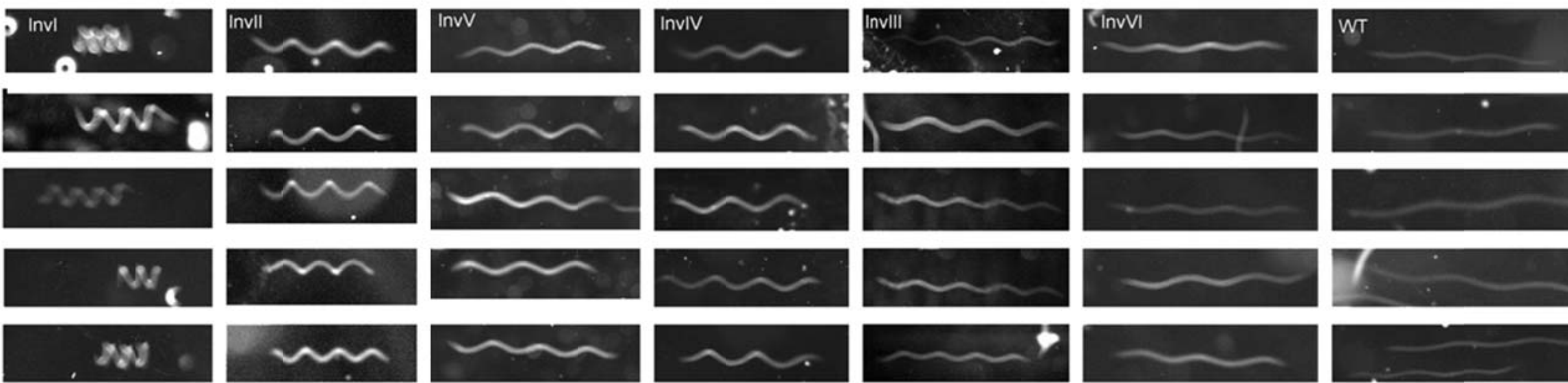

Fig. 12: Representative swimming trace images of the sublines shown in fig. 11 (in the same order: decreasing average number of inverted rows, left to right).

Figs. 11 and 12 show a diverse array of cortical and swimming phenotypes, all derived from the same progenitor cell, and thus genetically identical. In general, as the average number of inverted rows decreases, the average velocity and pitch of the coiled swimming path increase, while the diameter of the coil/helix decreases. This reinforces the hypothesis that variants with fewer inverted rows display more efficient swimming (approaching the WT pattern), which is in all likelihood the foundation of the differential proliferative success and suppression of mean inversion size seen in Figs. 5 and 6. As variants approach wild type swimming, their ability to capture food and maneuver more efficiently most likely provides them with a proliferative advantage over cells with a greater number of inverted rows.

CONCLUSIONS

Here we have demonstrated that cortical inversions in *Paramecium* can undergo spontaneous changes that directly affect cellular swimming pattern, and that both changes in corticotype and swimming phenotype can be accurately followed at the population level with quantitative methods. We conclude that cortical rearrangements in cells with inversions are not only possible, but commonplace. The cortical variants generated by these rearrangements exhibit diverse swimming phenotypes with respect to the parameters shown in fig. 11. As expected, cells with fewer inverted rows swim faster and in a straighter path (decreasing helical diameter) with fewer helical turns per unit time, and we assume that this gives them a selective advantage – especially in mass culture, in which competition for bacteria quickly becomes a significant influence on population dynamics.

The epigenetic inheritance of cytoskeletal rearrangements in the cortex, and their corresponding effect on swimming pattern, reaffirm that many aspects of cellular phenotype are not under direct genomic control (Kacser and Small 1996). The transmission of heritable structural information in the cortex is an emergent property of the geometry of preexisting cortical structures and the nature of basal body/cortical unit replication. Morphogenesis and cortical patterning thus depends not only on the expression of nuclear genes (and limited processes of self-assembly of these gene products), but additional, non-genic forms of information "encoded" in the arrangement of parental structures.

Further, microscopic observations have suggested that some aspects of global patterning are disrupted in cells carrying cortical inversions. Inverts with more than a few inverted rows frequently have a chaotic placement of cortical units in the anterior polar region. These cells also tend to exhibit extreme ciliary row distortion/bending in the inverted region near the future division furrow, potentially as a result of weakened or disturbed cytoskeletal rearrangements in this region during morphogenesis. Broken or shortened ciliary rows are common, and some of these may be the product of this extreme row distortion at some point after cell division. Some of our observations also indicate that both normally-oriented and inverted rows can rotate 180° in the poles of the cell and potentially telescope between adjacent rows (see fig. 8), thereby adding a "new" row in the opposite orientation. Overall, it is clear that cell populations with cortical inversions do not retain a constant average corticotype, unless stringent selection of swimming types is periodically applied. The molecular basis of these spontaneous morphogenetic errors and rearrangements is entirely unknown. Nonetheless, it is clear that the nature of structural inheritance makes cortical rearrangements persistant features of clonal offspring, and produces the heritable variation required for biological selection to act.

In the simple experiments shown in figs. 5 and 6, it is evident that selection has acted to destroy the inverted phenotype. In this context one might consider spontaneous changes that increase the number of inverted rows in the same light as deleterious genetic mutations. This subsequently demands consideration of the opposite possibility: can exceedingly rare but beneficial rearrangements to the cortex appear and become established, just as some spontaneous mutations can create better proteins? We have already observed that, in InvE, the inversion now seems to be separated into multiple domains, each with its own set of "wide" and "narrow" boundary

junctures, with the domains separated by an intervening row (or rows) in the normal orientation. This was not the case when the InvE was first established, and so is yet another example of quasi-permanent, heritable restructuring of the cortical pattern. Although highly speculative, it must be asked whether the ciliate cortex presents an expanded set of options with regards to evolutionary adaptability – that is, whether some heritable alterations to the cortex might allow for the organism to better survive and proliferate, without necessitating genomic changes.

Future goals

Utilizing the simple method of quantitatively describing swimming parameters in cortical variants presented here, we believe it is possible to further develop a mathematical relationship and predictive model of swimming in *Paramecium tetraurelia*. The model would assume a circular, left-handed helical swimming path and define the simple parametric functions that describe a helix in terms of the number of inverted ciliary rows. Such a model would be a stepping stone for a better understanding of the hydrodynamics of ciliate swimming, namely the hydrodynamic efficiency of wild type swimming compared to the more coiled swimming in inverted cells.

REFERENCES

Aufderheide K.J. (2008): An overview of techniques for immobilizing and viewing living cells. Micron. 39:71–76.

Aufderheide K. J., J. Frankel, and N.E. Williams. (1980): Formation and positioning of surface related structures in protozoa. Microbiol. Revs. 44:252–302.

Aufderheide K.J., T.C. Rotolo, and G. W. Grimes (1999): Analysis of inverted ciliary rows in *Paramecium.* Combined light and electron microscopic observations. Europ. J. Protistol. 35: 81–91.

Beisson J. and Sonneborn T.M. (1965): Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia.* Proc. Natl. Acad. Sci. USA 52: 275-282.

Beisson J. (2008): Preformed cell structure and cell heredity. Prion 2: 1–8.

Beale G. H. (1954): The genetics of *Paramecium aurelia.* Cambridge University Press, London.

Dippell R. V. (1968): The development of basal bodies in *Paramecium.* Proc. Natl. Acad. Sci. USA 61: 461–468.

Fernandez-Galiano D. (1976): Silver impregnation of ciliated protozoa: procedure yielding good results with the pyridinated silver carbonate method. Trans. Amer. Micros. Soc. 95: 557–560.

Fernandez-Galiano D. (1994): The ammoniacal silver carbonate method as a general procedure in the study of protozoa from sewage (and other) waters. Wat. Res. 28: 495-496.

Foissner W. (1991): Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. Europ. J. Protistol. 27: 313–330.

Grimes G. W. and Aufderheide K.J. (1991): Cellular Aspects of Pattern Formation: The Problem of Assembly. *Monographs in Developmental Biology,* Vol. 22, Karger, Basel.

Iftode F. and Adoutte A. (1991): Un mecanisme de regulation morphogenetique chez Paramecium: la rotation du cortex. C. R. Acad. Sci. Paris 313: 65–72.

Iftode F., Fleury-Aubusson A. (2003): Structural inheritance in Paramecium: ultrastructural evidence for basal body and associated rootlets transmission through binary fission. Biol. Cell. 95: 39–51.

Jerka-Dziadosz, M., and J. Beisson, (1990): Genetic approaches to ciliate pattern formation: from self-assembly to morphogenesis. Trends Genet. 6: 41–45.

Kacser H. and Small J. R. (1996): How many phenotypes from one genotype? The case of prion diseases. J. Theoret. Biol. 182: 209–218.

Nanney, D. L. (1967): Cortical slippage in *Tetrahymena*. J. exp. Zool. 166: 163–170.

Rotolo, T.C., Grimes G. W. and Aufderheide K.J. (1995): The ultrastructure of "inverted" kineties in *Paramecium*. J. Euk. Microbiol. 42: 17A.

Sonneborn, T.M. (1964): The differentiation of cells. Proc. Natl. Acad. Sci. USA 51: 915–929.

Sonneborn, T.M. (1970a): Determination, development, and inheritance of the structure of the cell cortex. Symp. int. Soc. Cell Biol. 9:1–13.

Sonneborn, T. M. (1950): Methods in the general biology and genetics of *Paramecium aurelia.* J.Exp. Zool. 113: 87-148.

Sonneborn, T.M. (1970b): Methods in Paramecium research. Methods Cell Physiol. 4: 241–339.

Sonneborn, T.M. (1975): Positional information and nearest neighbor interactions in relation to spatial patterns in ciliates. Ann. Biol. 14: 565–584.

Sperling L., G. Keryer, F. Ruiz, and J. Beisson. (1991): Cortical morphogeneis in Paramecium: a transcellular wave of protein phosphorylation involved in ciliary rootlet disassembly. Dev. Biol 148: 205-218.

Tamm, S. L., T. M. Sonneborn, and R. V. Dippell. (1975): The role of cortical organization in the control of ciliary beat in *Paramecium.* J. Cell Biol. 64: 98-112.