

MORPHOLOGICAL VARIATION OF THE AFD CELL DENDRITE IN
GENETICALLY IDENTICAL *CAENORHABDITIS ELEGANS*

by

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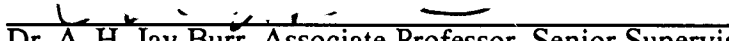
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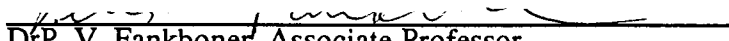
**MORPHOLOGICAL VARIATION OF THE AFD CELL DENDRITE
IN GENETICALLY IDENTICAL *CAENORHABDITIS ELEGANS***

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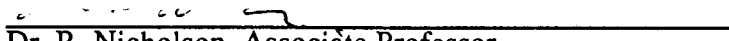
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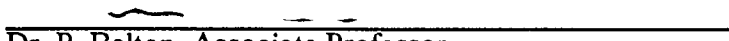
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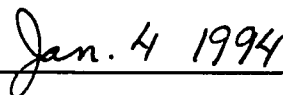


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Abstract

How much developmental variation or “noise” in the morphology of a cell can one expect between or within genetically identical individuals? I examined this in the amphidial finger dendrites (afd’s) of the nematode *Caenorhabditis elegans*. The afd’s have a modified cilium and numerous anteriorly and posteriorly projecting microvilli. I compared the number of afd microvilli from wild-type hermaphrodites of the N2 strain. These were raised to the same age and held at the same humidity at 18° C. Details of the morphological differences were revealed by reconstructing the left and right afd’s of one worm. Most microvilli originate from processes that project from the lateral surfaces of the terminal enlargement of the dendrite. These meander and sometimes reverse their direction of travel before branching into two or more microvilli, which may travel in opposite directions. Once formed, the majority of the microvilli consistently travel either anteriorly or posteriorly, and variation appears to be more in the number and length of the microvilli than in their guidance.

The microvilli of 83 afd’s were counted in transverse sections through fixed reference points. The mean number of anterior and posterior microvilli was 42.1 and 43.4; the difference was not statistically significant. The mean length of the cilium was 910 nm. What was interesting was the large variation. The range in number of anteriorly projecting microvilli was 23 - 60 and that of the posteriorly projecting microvilli was 36 - 53 while the range in the lengths of the cilia was 490 - 1470 nm. In ten worms for which all the microvilli of both afd’s could be counted, the numbers in left and right afd’s were correlated [$r=0.511$, d.f.=15, $P=.02$]. This indicates that part of the variation is due to differences among worms. Analysis of variance with a split-plot design revealed that an approximately equal contribution of variation is found between left and right afd’s within

worms as among the isogenic worms [variance (between) = 31, variance (among) = 24]. This may reflect developmental noise in the gene-controlled processes of development.

In addition to the morphological variation of the afd's, I investigated the possibility that the afd's may have a photoreceptive function since their numerous parallel microvilli resemble the light gathering organelle of many invertebrate photoreceptors.

Photoreceptive microvilli act to increase the surface area of the photoreceptive membrane and thus increase the likelihood of photon capture. Degradation and turnover of invertebrate photoreceptive microvilli is commonly observed to be affected by light conditions, and in some cases the development of invertebrate photoreceptors is stimulated by light. I raised the nematodes under either continuous illumination or continuous darkness, and serial transverse sections through either the light or dark raised afd's revealed no apparent signs of microvillar degradation. Statistical analysis of the number of afd microvilli of the light and dark raised nematodes revealed no significant difference between them. This indicates that if there are any effects of light or darkness on afd development it is not large enough relative to the background variation to be detected with a sample size of 83 afd's.

Dedication

I dedicate this work to the Lord, without whom none of this would be possible, and to my parents, for instilling in me the importance of education and hard work.

Acknowledgments

I wish to express my appreciation to the following people: Dr. Victor Bourne for his advice and guidance of the electron microscopy and photography; Dr. David Eaves and Francois Bellavance for their assistance and advice on statistics; Drs. Peter Fankboner and Russell Nicholson for their helpful comments and suggestions; Elizabeth Carefoot for her assistance with the diagrams; Gloria Allende and my friends at S.F.U. who encouraged me and made this experience enjoyable. I particularly thank Dr. A. H. Jay Burr, my senior supervisor, who has encouraged and facilitated this work in every possible way; and Bonnie, my wife, for her patience, support and enthusiasm throughout this project.

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Chapter 1

GENERAL INTRODUCTION

Developmental processes are responsible for the formation of all biological material, from organelle to organism. As molecular techniques continue to be refined, so does our interest in and knowledge of the control of such processes. Ultimately, the control of development resides in the control of gene expression. Regulation of DNA transcription was first demonstrated at the molecular level by Hayashi et al. (1963) for the lactose operon in *E. coli*. Since then, much more information regarding the control of protein synthesis has been elucidated in both prokaryotes and eukaryotes. It is now known that protein synthesis of eukaryotes can be controlled at five main levels: (1) transcriptional control, which controls how, when, or in which cell a given gene is transcribed; (2) processing control, which controls how the initial mRNA transcript is processed; (3) transport control, which selects the completed mRNAs in the cell nucleus which are to be exported to the cytoplasm; (4) translational control, which selects the mRNAs in the cytoplasm which are to be translated by the ribosomes; (5) mRNA degradation control, which selectively stabilizes certain mRNA molecules in the cytoplasm. Depending on the protein, and the cell in which it is produced, the control of its synthesis may occur at any or all of the above steps (e.g. Derman et al., 1981; Gliniak and Rohrshneider, 1990; Hill and Morris, 1993; Kabnick and Housman, 1988).

Molecular techniques may provide us with the means to determine the step or steps in the developmental pathway at which the control is achieved, however, determination of the degree of precision to which the control mechanisms are regulated is beyond the limitations of the current molecular techniques. Until the advancement of molecular techniques enables us to precisely quantify the contributions and interactions of the developmental control mechanisms, a detailed analysis of the phenotypic variation of biological structures among isogenic organisms may be the only means of providing us

with a basic understanding of the limits of precision of the developmental processes. One of the purposes of this thesis is to provide a detailed analysis of the structure of the amphidial finger dendrites (afd's) in an isogenic strain of the nematode *Caenorhabditis elegans*, and to determine the amount of phenotypic variation among them.

Phenotypic variation among individuals of the same species is normally attributed to genetic polymorphisms or environmental differences during development. Phenotypic variation among genetically identical organisms raised under identical conditions, however, indicates that there is another component contributing to the variation which was termed “developmental noise” by Waddington (1957). Developmental noise is apparently the outcome of limited precision in the control of development or gene expression. At this time, however, there is no evidence of where in the developmental pathway, or at which level of control of gene expression, this lack of precision could occur.

Phenotypic variation among genetically identical organisms has been illustrated within several species of invertebrates. Macagno et al. (1973) illustrated a classic example of developmental noise by revealing the variation in synaptic and branching patterns of identifiable neurons among isogenic individuals in the crustacean *Daphnia magna*. Their experiment was the first to use isogenic organisms in order to study the constancy and variability within the nervous system, and they concluded that the overall shape, location and branching patterns of the fiber terminals are variable, and “...seem to reflect the noise level of the genetic control of structure”. Similar studies by Goodman (1978) also revealed a considerable degree of variability in soma location and branching patterns of identified neurons in isogenic locusts of the genus *Schistocerca*. These studies support the hypothesis that non-genetic factors, such as developmental noise, contribute to phenotypic variability. Other important reasons for studying variability is that it can provide further insight into the mechanisms of development and evolution of the nervous system. Furthermore, a detailed knowledge of the variation in the structure of single neurons may

provide an understanding of the variability of some aspects of behavior. My detailed analysis of phenotypic variation among the afd's of *C. elegans* lays the groundwork for studies of developmental noise in this organism which has major advantages for using genetic and molecular approaches.

Since 1965 *Caenorhabditis elegans* has become increasingly popular as a model animal to investigate the genetic, ultrastructural¹, and behavioral aspects of development and function in a simple nervous system. Initially chosen for its suitability for rapid genetic and biochemical analysis, *C. elegans* has proven to be valuable as a model system for scientific study. It is a relatively simple organism, both anatomically and genetically, and its population consists of two sexes; hermaphrodite and male. The hermaphrodites produce both oocytes and sperm, and therefore may self-reproduce. The males which occur at relatively low frequencies (0.1%), have the capacity to fertilize hermaphrodites which allows genetic crosses to be constructed. Each hermaphrodite can lay up to 300 eggs, each having an incubation time of about twelve hours. Upon hatching, the larvae develop through four stages before becoming adults. The length of the adult worm is about 1 mm, and is thus conveniently handled in large numbers. The total number of cells of the worm is only 959 in the adult hermaphrodite, and 1031 in the adult male. Although this is a relatively small number of cells, this organism is well differentiated and has an intestine, epidermis, excretory system, muscle and nerve cells as well as reproductive organs. Relative structural simplicity, along with differentiation, ease of cultivation, a short reproductive cycle, and manageability in large numbers makes *Caenorhabditis* a model organism for diverse scientific analysis. The small size also makes the worm quite amenable to complete anatomical studies at the electron microscope level.

Much work has already been done to describe the structure of the nervous system of *C. elegans* (e.g. Chalfie and White, 1988; Lewis and Hodgkin, 1977; Perkins et al., 1986; Ward et al., 1975; White et al., 1986). Of the 302 neurons which comprise the entire nervous system of the hermaphrodite, the anterior sensory nervous system, which

includes the two afd's, accounts for 58. The afd's are located in the amphids: two large antero-lateral sensilla, each containing the dendrite endings of 12 sensory neurons and two non-neuronal cells (Fig. 1). Most of the dendrite endings of these neurons each contain a single ciliary process, however, two of the channel neurons each contain a pair of cilia. The non-neuronal cells, the sheath cell and socket cell, form a channel connected to the exterior. Many of the ciliary processes project into the amphidial channel. The others, including the afd cilium, invaginate the sheath cell. The space surrounding the sensory endings is sealed off from the space around the dendrite process by tight junctions (Fig. 1). The axons of these sensory neurons synapse just posterior to the nerve ring of the nematode.

The amphid cilia like all sensory cilia in nematodes are obviously modified in many ways from that of a typical kinocilium (Ringo, 1967). They have no apparent basal bodies (Wright, 1980), however, the proximal segment of these cilia contains a transition zone (Fig. 1). This zone comprises nine doublet microtubules joined to the membrane by Y-shaped cytoskeletal links (Gilula and Satir, 1972) and are drawn inward by attachments to a central cytoskeletal cylinder (Fig. 2). Perkins et al. (1986) suggest that the central cylinder may correspond to the apical rings found in the transition zones of motile cilia in some organisms. Two other segments of the cilia are easily observed. The middle segment lacks the central cylinder and the doublets spread apart and become less organized. The distal segment of the cilia contains only the A subfibers of the doublet microtubules and the inner singlet microtubules.

The socket cell, which wraps around and seals to itself via a tight junction, creates the channel and attaches the amphid to the hypodermis (Fig. 1). The other non-neuronal cell, the sheath cell, is a glial-like cell which envelops the dendrite endings of the sensory neurons. The sheath cell contains a large golgi apparatus and gives rise to many large

Fig. 1. Schematic longitudinal section through amphid sensillum in wild-type *C. elegans*. The amphid channel (ac) is formed from a socket cell (so) and a sheath cell (sh). The socket channel is lined with cuticle that is continuous with the external cuticle. The dendrites of three channel neurons and one wing neuron (AWA) are shown. The AFD dendrite remains separate from the fascicle of the wing and channel cilia. For the AFD dendrite the microvilli (m), the cilium (c), and the transition zone (TZ) are indicated. Tight junctions are seen just before where the dendrite processes differentiate into the sensory endings. Modified from Perkins et al. (1986).

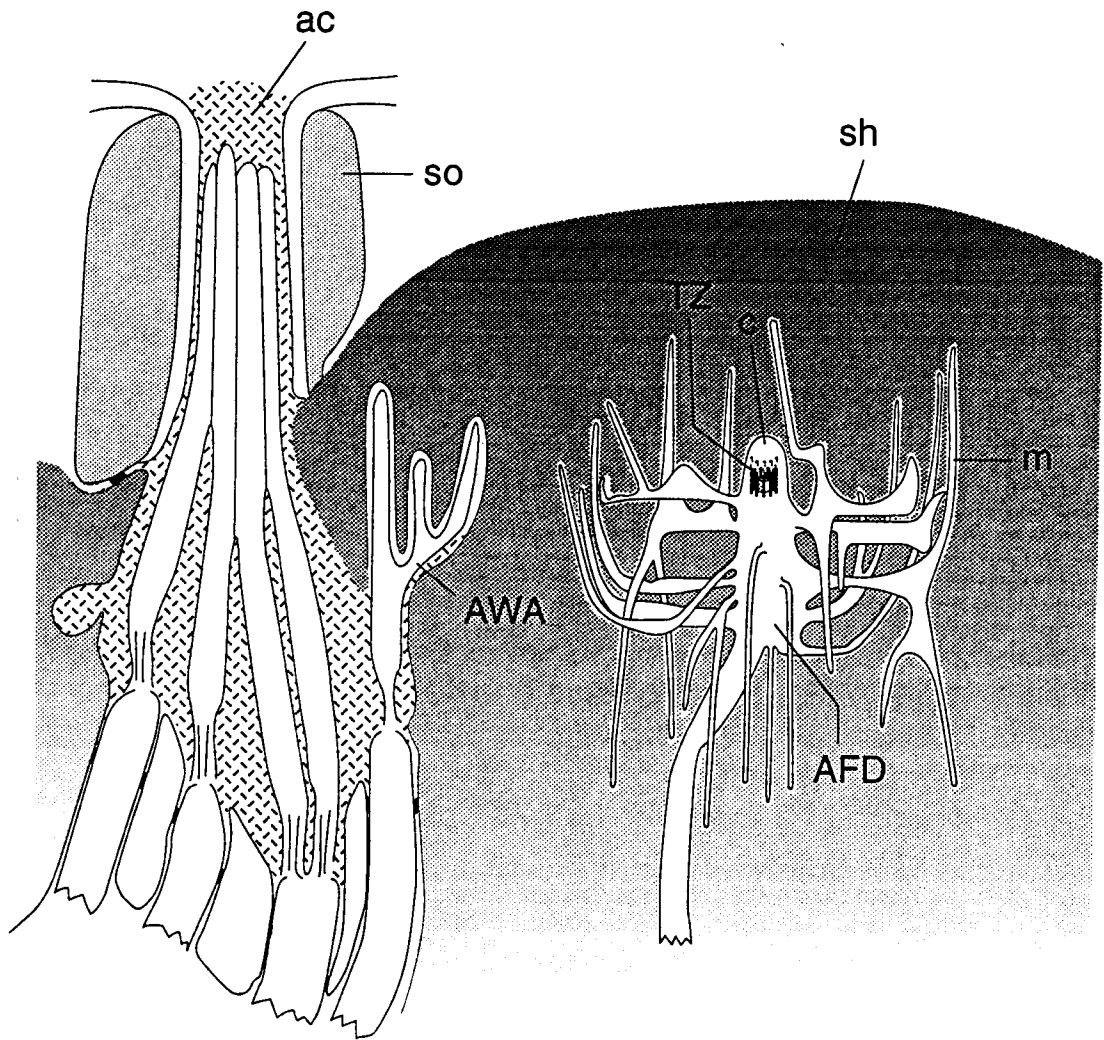
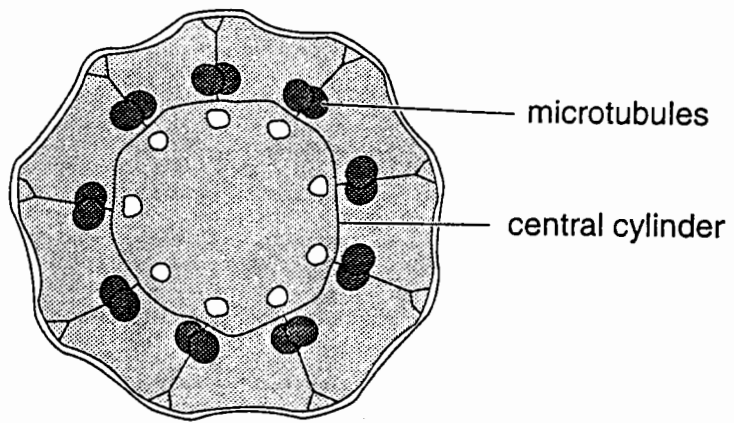


Fig. 2. Transition zone of afd cilium showing the central cylinder and the 9 + 0 arrangement of microtubules.



vesicles along its concave surface facing the channel. The function of the sheath cell is unknown, however it is thought to regulate the external chemical environment of the sensory ciliary processes by secreting material into the amphid channel (Chalfie and White, 1988).

Eight of the sensory neurons are contained within the amphidial channel, and therefore are accessible from the external aqueous environment and thus may have an olfactory function. The other four sensory neurons have unusually shaped endings which do not travel entirely up the amphidial channel (see Ward et al., 1975). For the three “wing neurons”, the awa, awb, and awc, the dendrites travel within the amphidial channel to the level of their ciliary transition zones. At this point the cilia invaginate the sheath cell from inside the lumen of the amphidial channel and form wing-like projections. The dendrite of the afd neuron, on the other hand, penetrates the sheath cell without passing through the channel (Fig. 1). The microvillous ending of the afd cell thus is not connected to the amphidial channel. It has only a rudimentary cilium and its apical membrane expands into numerous microvillous “finger” projections which invaginate the sheath cell.

The morphology of the afd's of *C. elegans* appears homologous to the microvillous dendrites of amphidial sensory neurons commonly found in other nematodes throughout the classes secernentia and adenophorea (Coomans, 1979). The small size of these nematodes prevents electrophysiological studies of the sensory neurons, however, other approaches such as laser ablation have provided evidence of olfactory function for most of the amphidial sensory neurons (Bargmann, 1993). The eight ciliated amphidial sensory neurons that are exposed to the exterior environment of the nematode have been identified, through laser ablation experiments, as chemosensory neurons sensitive to soluble odors.

Evidence from behavioral mutants of *C. elegans* suggests that the afd's may act as thermoreceptors (Perkins et al., 1986). Also, unpublished findings by Mori, I., Honda, H., and Ohshima, Y. (Kyushu University) lead to the conclusion that the afd's play a critical

role in thermotaxis. Their preliminary results indicate that laser ablation of only the *afd* neurons generates a cryophilic phenotype in wild-type *C. elegans*. *Caenorhabditis*, as well as a number of plant parasitic nematodes, are known to re-locate to the temperature at which they were raised, their acclimation temperature, when exposed to thermal gradients (see El-Sherif et al., 1969; Hedgecock and Russell, 1975). *Caenorhabditis* behavioral mutants such as *ttx-1*, however are cryophilic and always seek colder temperatures regardless of their thermal history. *Ttx-1* is also hyper-responsive to dauer-inducing pheromone, which is produced during periods of overcrowded conditions or starvation (e.g. Golden and Riddle, 1984). Wild-type *C. elegans* is more responsive to pheromone at temperatures above 25° C, thus *ttx-1* individuals appear to be defective in temperature sensitivity. The *afd*'s from the cryophilic *ttx-1* mutants lack the numerous microvillous projections which are characteristic of the wild-type *afd*'s, therefore, the structure of the *ttx-1* *afd*'s resembles that of a fingerless sack of membrane which protrudes from the dendrite just below the cilium (Perkins et al., 1986). In addition, the *afd* cilia of the *ttx-1* mutants are different from those of the wild-type animals. The mutant cilia are approximately three times longer than their wild-type counterparts, and instead of projecting directly anterior the *ttx-1* cilia are tilted ventrally at their bases. The cilia of the other neurons associated with the amphid, the wing and channel cilia, appear normal in *ttx-1* mutants.

Genetic analysis has also revealed that at least 14 genes can be mutated to produce chemotaxis defective mutants. Of these, *tax-2,3,4* and *6* are also thermotaxis defective. This genetic overlap between chemotaxis and thermotaxis is thought to be caused by common gene products in different sensory neurons, or by a common motor pathway in thermotaxis and chemotaxis (Hedgecock and Russell, 1975). Evidence presented by Hedgecock and Russell (1975), however, indicates that the *ttx-1* gene is not essential for normal chemotaxis.

Several nematode sensory organs have previously been reported to have a dual or bimodal function. Burr and Burr (1975) present evidence indicating that the amphid of the nematode *Oncholaimus vesicarius* functions bimodally in both chemoreception and photoreception. In addition, a combined chemosensory and mechanosensory function has been suggested for the spicular sensillum of *Pratylenchus* (Wen and Chen, 1976) and *Aphelenchoides* (Clark and Shepherd, 1977), however, *Aphelenchoides* has only one dendrite in each spiculum to achieve this combined function. In accordance to this, one could speculate that the afd may function as a bimodal sensory cell. It contains both a cilium and microvilli and it may be sensitive to more than one modality of stimulus. The evidence discussed above implicates the function of the afd as thermoreceptive. In addition, certain morphological characteristics of the afd cell, namely the numerous microvillous projections, appear analogous to the microvillar (rhabdomeric) photoreceptors of a host of other invertebrates, most notably to those within the phyla mollusca, annelida, and arthropoda (reviewed by Burr, 1984). There is also a similarity in structure between the afd cell and the photoreceptor organelles in the eyes of some turbellarians and rotifers. Furthermore, Burr (1985) demonstrated that *C. elegans* exhibits a weak photoresponse: an increase in the probability of a reversal, whereby the animals temporarily backed up. Coomans and DeGrise (1981) suggests that this type of photoresponse could be particularly beneficial to soil nematodes, such as *C. elegans*, as it would enable the animals to avoid the unfavorable conditions at the surface. Part of my work, therefore, was focused on finding evidence for a photoreceptive function of the afd microvilli.

The morphology of the microvillar photoreceptors varies slightly among the different phyla with respect to the degree of microvillar ordered arrangement, the position of the microvilli, and the size and number of the microvilli. A common theme among all of them, however, is that the photoreceptive membranes are continually being shed and renewed in a process of membrane turnover. In many cases light triggers the process. The

effect of light on membrane turnover of photoreceptive microvilli differs among the invertebrate phyla. Exposure to intense illumination or continuous darkness induces an instability and disintegration of the photoreceptor microvilli of various invertebrates (e.g. Behrens and Krebs, 1976; Blest and Day, 1978; Brammer et al., 1978; Eguchi and Waterman, 1979). In many organisms, such as crabs, spiders, *Limulus*, and *Daphnia*, even normal daylight intensities have been shown to induce structural changes in the photoreceptors by causing a disintegration in the number and ordered array of the microvilli (e.g. Blest, 1978; Herman, 1991; Rohlich, 1967; Stowe, 1980). Subsequent exposure to darkness increases the membrane surface area and returns the microvilli to their ordered array. This light induced breakdown of the microvilli results in an increase in multi-lamellar bodies, vesicles, and debris, and has been shown to correlate with an increase of lysosomal activity (e.g. Brandenburger and Eakin, 1985).

In another example, light appears to induce the formation of the microvillar structure. The photoreceptive microvilli of the turbellarian flatworm *Dalyelliidae* degenerate in the dark and regenerate in the light (Bedini et al., 1977). The light adapted microvilli are in a parallel array, their profiles are regular, and the tips are rounded. The microvilli lose their structure in the dark, they lack their straight arrangement, and the tips become curly with irregular foldings. Bedini et al. (1977) have shown these microvilli to be completely reconstructed within five to ten minutes of exposure to light after 12 hours of darkness. Another example which indicates the varying effects of light on photoreceptive membrane turnover is seen in vertebrate rods and cones. Young (1978) investigated the effects of light and dark on the rods and cones of *Gallus domesticus* chicks, and found that shortly after the beginning of a 12-hour light period the rod outer segments sloughed off the distal tips, including groups of the membrane disks. Early in the 12-hour dark period, however, the cone outer segments discarded groups of their membranes. These results perfectly complemented those obtained earlier by O'Day and Young (1977) in a goldfish, and by Young (1977) in a lizard, and suggest that

photoreceptive membrane turnover in vertebrates oscillate with a daily rhythm which is synchronized with the daily fluctuation of light in the environment. One of the aims of my work is to determine whether light influences the microvillar structure of nematode and dendrites.

The function of a sensory structure is sometimes implied by its morphology or its relationship with other cells. For example, to serve efficiently as chemoreceptors, the membrane surfaces of the chemoreceptors must be accessible to diffusing odorant molecules. This means that the sensory nerve endings should be in contact with the exterior environment of the animal, an example of this is the nematode amphidial neurons which are located in a channel that is continuous with the exterior environment of the nematode. For photoreception there are obviously different requirements, and in most cases the photosensitive membranes of animals have an increased surface area to maximize the likelihood of capturing a photon. Vertebrate rod and cone cells, for example, are composed of numerous flattened membrane discs. Likewise, numerous microvilli increase the surface area of many invertebrate rhabdomeric photoreceptors.

Rhodopsin is the photoexcitable pigment molecule embedded in the photoreceptive membranes of animals. It consists of an opsin protein and a retinaldehyde chromophore, and is extremely stable in the absence of light. In the presence of light, the absorption of a photon by the rhodopsin causes the molecule to undergo a series of transformations which initiate a cascade of reactions leading to the excitement of the photoreceptive membrane (e.g. Stryer, 1988; Detwiler and Gray-Keller, 1992). This process of phototransduction, whereby the capture of a photon of light triggers a cascade of chemical reactions and modulation of ion channels, leads to hyperpolarization of the phototransductive membrane in the case of vertebrate rod and cone cells (e.g. Detwiler and Gray-Keller, 1992), or depolarization of the phototransductive membrane in the case of invertebrate ciliary or rhabdomeric photoreceptors (e.g. Tsuda, 1987). Previous intensity-response results indicate that *C. elegans* lacks a significant response to illumination in the range of 420-440

nm, and has a statistically significant response to illumination in the range of 520-600 nm (Burr, 1985). These results rule out hemoglobin, carotenoids or flavin, which were previously thought to be the nematode photopigment, and Burr (1985) suggests that a rhodopsin is the best candidate for a photopigment in *C. elegans*.

The initial stages of my research were focused on determining whether the afd dendrites serve a photoreceptive function. To determine whether the afd microvilli of *C. elegans* are involved in photoreception, I analyzed the microvilli for any morphological changes resulting from illumination or darkness. As described above, these conditions affect the microvilli of all invertebrate photoreceptors, and due to the small size of this nematode, more direct methods of determining the true function of the afd microvilli, such as electrophysiology, are not possible at this time. Also, isolation of photosensitive mutants are impossible due to the weak, statistical nature of the response. As well as looking for light effects, I have revealed a more detailed view of the afd morphology by completely reconstructing two afd's from serial electron micrographs. These reconstructions helped to clarify some differences between my data and the previous reconstruction of the afd cell by Ward et al. (1975). Finally, the remainder of my work was focused on the causes of variation that I observed in the number of microvilli among afd dendrites of many worms. From these analyses, I have determined that there is a significant amount of additional variation among afd dendrites from wild-type worms that is not accounted for by any of the observed variables or experimental treatments. I propose that this additional variation may be a result of developmental noise.

General Materials And Methods

Nematode Cultures

Wild-type N2 hermaphrodites of the nematode *C. elegans* (var. Bristol), obtained recently from the *C. elegans* genetics center, were raised at room temperature (18° C) on agar petri plates seeded with *E. coli* (Brenner, 1974). Nematode cultures were initiated by choosing one egg and placing it on a fresh petri plate for the purpose of isolating an individual nematode. The individual nematode was then raised to the mature egg laying adult stage and was permitted to lay approximately 50 eggs before it was removed from the plate. The eggs were then collected into two groups, and each group was then transferred to a fresh agar plate. These two groups of eggs were then placed inside specially designed growth containers (described below). One of these groups was then maintained under the condition of continuous illumination and the other was maintained under the condition of continuous darkness until the nematodes reached the one to two day old adult stage.

Growth Conditions

To facilitate the growth conditions of continuous darkness, a light-safe container was constructed from black, 0.25" Plexiglas. The main function of the container was to completely block the entry of light while allowing ventilation through a baffle, and it was designed to accommodate up to sixteen agar plates. The environmental conditions of the light exposed group of worms were maintained the same in a second growth container of the same design except made of transparent Plexiglas.

The two containers were placed adjacent to one another inside the same incubator where the temperature was maintained at 18° C. Two "cool white" fluorescent lamps, placed approximately 18 inches above the containers, were used as the source of illumination inside the incubator. The irradiance inside the transparent growth container

was 475 $\mu\text{W}/\text{cm}^2$, and the amount of radiation entering the opaque container was negligible (less than 0.02 $\mu\text{W}/\text{cm}^2$). Three inches of water was placed between the illumination source and the containers. This served to filter infrared radiation from the fluorescent illumination source, and to maintain a high humidity. The temperature within each container, measured with a YSI tele-thermometer, was 18 $^\circ$ C, and the difference in temperature between the containers was less than 0.1 $^\circ$ C, the precision of the tele-thermometer.

Fixation and Embedding

Worms were fixed in osmium/phosphate buffer. Glutaraldehyde was omitted from the fixation process because it darkened the cytoplasmic matrix and made it difficult to resolve membranes and cell boundaries. The fixation protocol was designed for serial reconstruction of *C. elegans* (e.g. Ward et al., 1975). Specimens were removed from the agar plates with a platinum pick and transferred to fixative (1% OsO_4 in sodium phosphate, pH = 7.4). Each nematode was immediately cut near the anterior tip. Nematode cuticle often prevents adequate diffusion of fixative and embedding material into the worm, therefore, an incision close to the site of interest allows a more rapid penetration and preservation of the target membranes. Dark-treated photoreceptors of some lower invertebrates regenerate rapidly upon exposure to illumination (Bedini et al., 1977). For this reason, I fixed the light-raised worms under illuminated conditions, and performed all microscopic manipulation and fixation of dark-raised worms under a Wratten series 2 safelight filter. Burr (1985) observed that *C. elegans* is insensitive to the wavelengths over 650 nm that are transmitted by this filter.

Specimens were fixed for one hour, then rinsed three times in 0.08M sodium phosphate buffer and placed on a thin layer (approximately 2 mm thick) of freshly poured agar (1% Noble). Each specimen was then gently pushed into the agar with a platinum pick, and small blocks of agar (2 mm x 2 mm), containing the specimens, were excised and

dehydrated in an ethanol series. They were then infiltrated through a graded series of propylene oxide before being transferred to a low viscosity embedding medium for the final infiltration (Spurr, 1969). The specimens were then transferred to fresh Spurr after 8 hours and placed into flat embedding molds. These molds permitted the specimens to be aligned with the anterior tip of the nematode perpendicular to the potential cut face of the block. They were then hardened overnight at 60° C.

Sectioning, Staining, and Electron Microscopy

Serial sections 65 nm thick (silver interference color) were cut with a diamond knife on a Reichert OM/1 ultramicrotome. About 200 serial sections, beginning at the anterior tip of each worm, were cut transverse to the long axis of each nematode and collected on Formvar coated (0.4%) slot grids. The 1 mm x 2 mm slot grids were carbon coated, prior to specimen collection, to add additional strength to the Formvar. The collected sections were stained with 5% uranyl acetate for ten minutes at room temperature then rinsed in twice distilled H₂O and transferred to Reynolds lead citrate for three minutes (Reynolds, 1963). Each grid was then rinsed one last time in twice distilled H₂O and placed in LKB 4828B specimen grid boxes. These grid boxes allow for the storage of grids in an ordered and easily identifiable manner. Electronmicrographs were taken on a Phillips 300EM at 60 to 80 KV.

Chapter 2

SERIAL RECONSTRUCTION OF TWO AFD'S

The complete reconstruction of a structure from serial electronmicrographs provides more information than individual thin sections. Previous reconstruction of the anterior sensory structures of wild-type *C. elegans* has provided a detailed description of the morphology of the anterior sensory structures in addition to their relationships with other cells (Ward et al., 1975). Understanding the morphology of “normal” sensory cells provides an important foundation from which anatomical lesions of behavioral mutants can be discerned.

The focus of this work is to determine whether morphological variation exists among wild-type afd's from genetically identical worms. The afd's may vary in their morphology within worms (between left and right afd's) and among like-sided afd's of different worms. In their reconstruction of the anterior sensory dendrites, Ward et al. illustrated the afd as possessing tightly packed and well organized microvilli of uniform size and shape. To determine if there was any variation in the number of afd microvilli, both within and among worms, I analyzed the number of afd microvilli from serial transverse electronmicrographs. This analysis indicated that the afd microvilli were much less organized and non-uniform than those previously reported by Ward et al. (1975). To determine the extent of the morphological differences between the afd's that I sampled and those previously reported by Ward et al., I completely reconstructed two afd's from transverse serial electronmicrographs of one nematode.

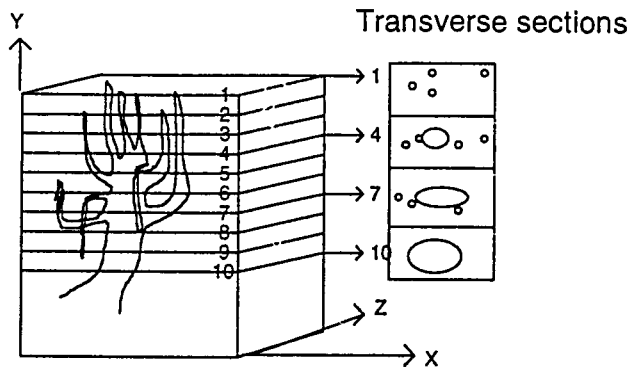
Methods

I reconstructed the left and right afd's of one nematode from serial electronmicrographs (12,000X). Color coding the microvilli in photocopies of the micrographs allowed the microvilli to be easily followed from section to section, and permitted the reconstruction of many microvilli at a time. The serial transverse sections were aligned using the cilium, the amphidial channel, and the triangular shaped lumen of the nematode as points of reference.

The reconstruction involved designating the thickness of each transverse section as the distance between the horizontal lines of graph paper. The horizontal lines of such graph paper thus represented a stack of serial transverse sections viewed from the side (Fig. 3). I scaled the Y-axis of the graph to 70 nm/line in order to represent the approximate thickness of a thin section, and I scaled the X-axis of the graph to 90 nm/line in order to fit the reconstructed afd's onto the page. I then drew the width's of the cilia, microvilli, and the distances that the microvilli traveled laterally from the dendrite enlargements, to scale. Representing a three dimensional structure in two dimensions poses some difficulty in the area of illustrating certain details according their relative Z-coordinates. Therefore, I did not illustrate the positions of the microvilli relative to one another in the dimension of the Z-coordinate of the sections. Illustration of all other aspects of the microvilli, such as the position of their origins, ends, branch points, and bends was easily accomplished. These characteristics of the microvilli may be recognized in the transverse sections, and are accurately recorded in the X-Y projection plane.

Fig. 3. Serial reconstruction of an afd dendrite

Stack of serial transverse sections
as viewed from the side



The transverse serial sections and graph lines are numbered accordingly, and reconstruction of each individual microvillus was initiated by locating the origin of the microvillus. The origin of a microvillus is easily detectable by the process of following the microvillus proximally through the serial sections to the location where the microvillus originates from the dendrite enlargement. Since the transverse sections were numbered, and these numbers corresponded to the Y-coordinate of the graph, the origins, branch points, and endings of the microvilli were easily recorded on the graphs. This created a two-dimensional reconstruction of the afd's as viewed from one side.

Results

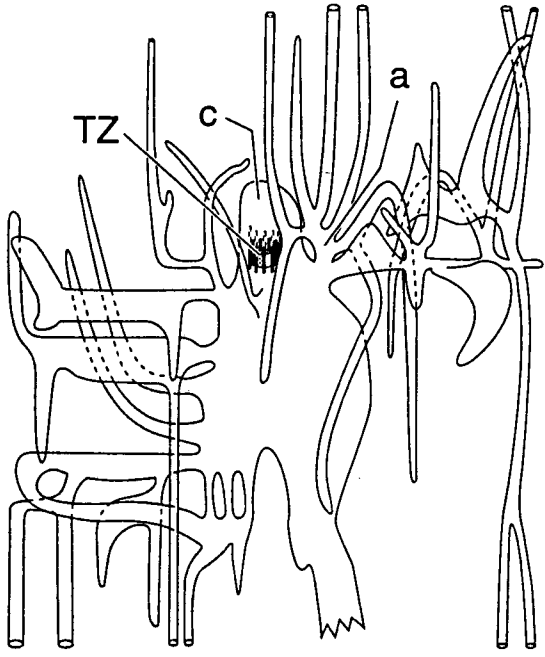
Figures 4-7 are four different views of the same right afd, and Figures 8-11 are four different views of the same left afd. Both afd's are from the same worm. The reason that four different views of each afd are illustrated is that there are approximately 100 microvilli on each afd, and not all of the microvilli could be accommodated in one two-dimensional drawing. Each of the four views of the afd's, thus illustrate approximately 25% of the total number of microvilli. Some of the general features of the afd cells that are illustrated in these figures include the cilium, numerous microvilli, the dendrite enlargement and the dendritic process which transmits signals from the dendrite to the nerve ring. Notice that most microvilli originate primarily from lateral processes of the dendrite enlargement.

Cilia

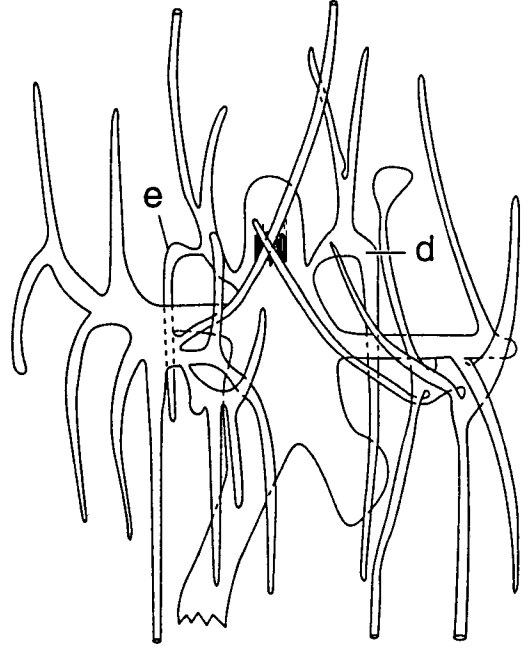
Each afd contains one anteriorly projecting cilium which emerges from the apex of the dendrite enlargement. The afd cilia generally contain a 9 + 0 microtubular arrangement, which differs somewhat from the 9 + 2 arrangement in motile cilia (reviewed by Alberts et al., 1983). The diameters of the cilia from the two reconstructed afd's are similar (approximately 0.75 μm), however the lengths of the two cilia differ by approximately 21% (Table's 1a, b). This difference in the length of the cilia is also seen in sampled afd's, and is another example of morphological differences among afd's.

Certain segments of the cilia permit the identification of several planes of reference in the transverse sections. These include the proximal segment (transition zone) of the cilia, the middle segment, and the distal segment. These are useful for identifying transverse sections through sampled afd's, and are discussed in detail in chapter three.

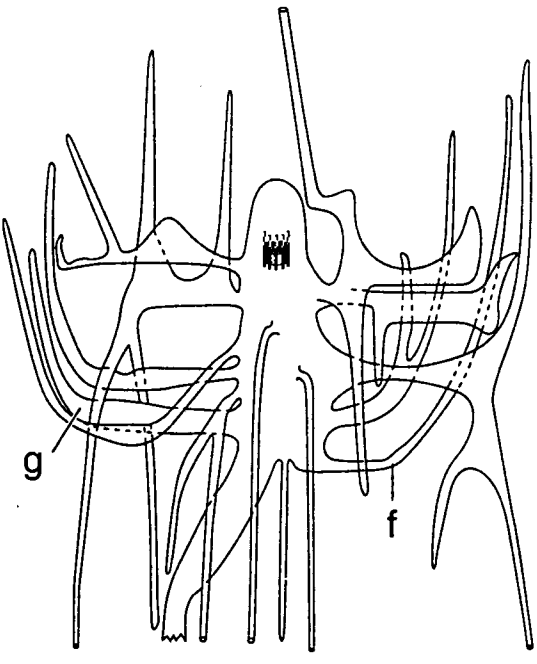
Fig's. 4-7. Reconstructed projections of the right-sided afd. Fig. 4: View number 1, note the lateral projection which branches into seven microvilli (a), the cilium (b), and the transition zone (c). Fig. 5: View number 2, note the microvilli that originate from the anterior of the dendrite enlargement and then travel posteriorly (d, e). Fig. 6: View number 3: note the microvilli that originate from the posterior of the dendrite enlargement and then travel anteriorly (f, g). Fig. 7: View number 4



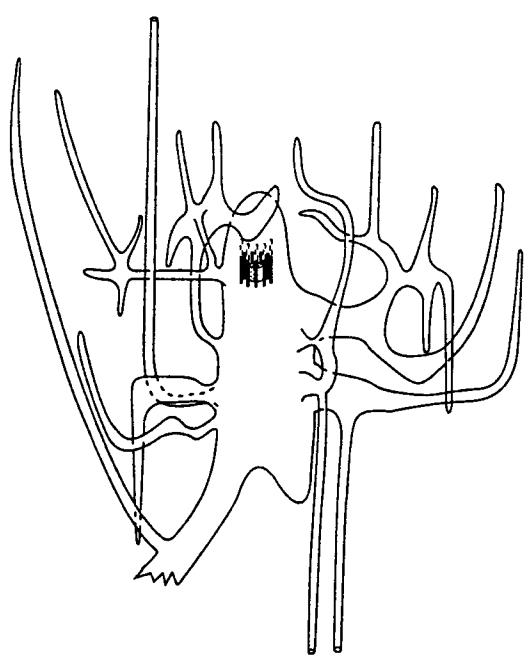
(4)



(5)

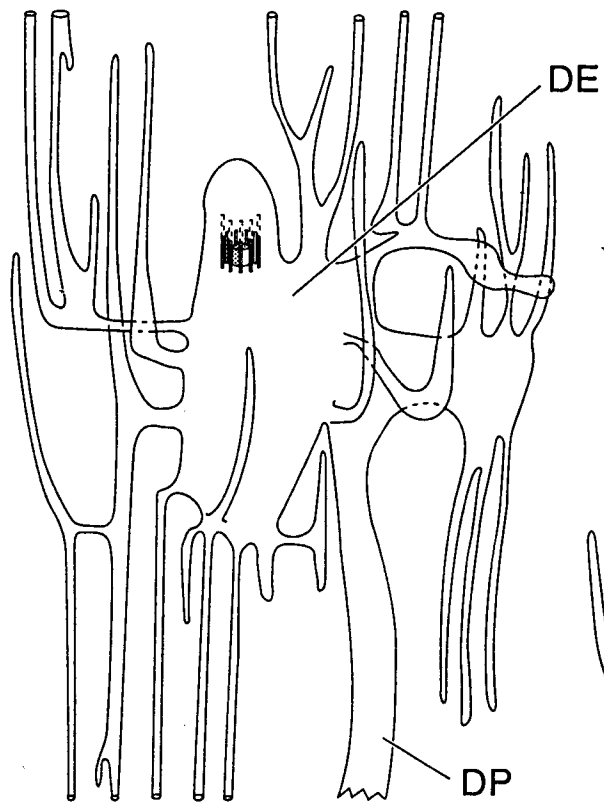


(6)



(7)

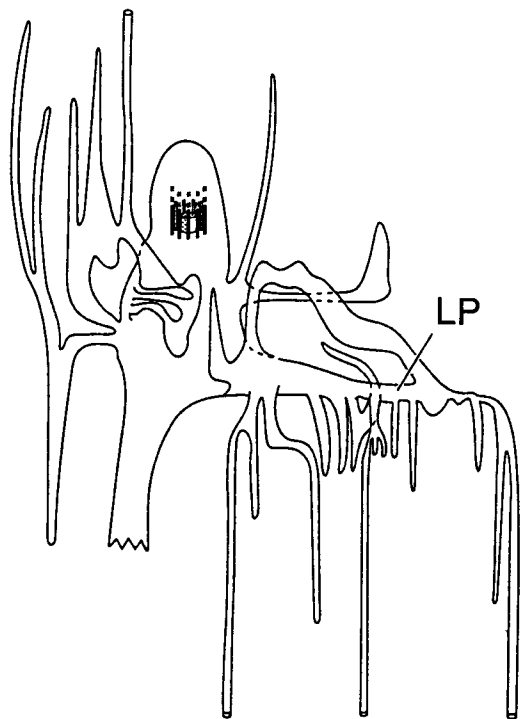
Fig's. 8-11. Reconstructed projections of the left-sided afd. Fig. 8: View number 1, note the dendrite enlargement (a) and the dendritic projection (b). Fig. 9: View number 2. Fig. 10: View number 3, note that many microvilli may originate from a single lateral projection (c). Fig. 11: View number 4



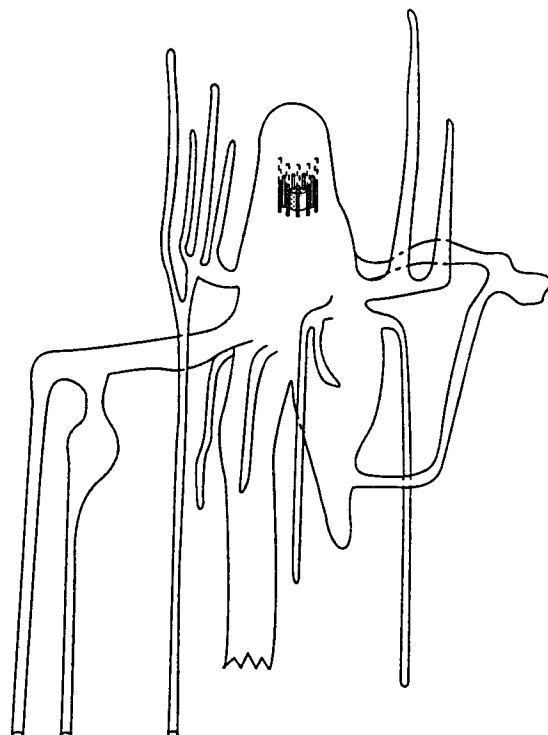
(8)



(9)



(10)



(11)

Table 1. Numbers of microvilli in the anterior regions of the left and right reconstructed afd's

a. Left afd: actual number of anteriorly projecting microvilli = 51, cilium length = 980 nm.

region	t. s. ^a	mean ^b	std. dev.	mean - actual ^c	sampling error ^d
1	4	35	1.7	-16	-31%
2	6	37	0.8	-14	-27%
3	4	37	0.6	-14	-27%
4	3	36	0.6	-15	-29%
2 + 3	9	37	0.9	-14	-27%

b. Right afd: actual number of anteriorly projecting microvilli = 61, cilium length = 770 nm.

region	t. s.	mean	std. dev.	mean - actual	sampling error
1	4	42	1.3	-19	-31%
2	5	43	1.6	-18	-30%
3	3	46	2.0	-15	-25%
4	3	43	2.0	-18	-30%
2 + 3	7	44	2.3	-17	-28%

^a number of transverse sections through a region.

^b mean number of microvilli per region.

^c the mean number of microvilli of that region minus the actual number of anteriorly projecting microvilli.

^d the sampling error of that region = [(mean - actual number) / actual number of anteriorly projecting microvilli].

Microvilli

Numerous microvilli, or microvillar-like projections, originate from the dendrite enlargement of the afd's, and I refer to them as either anteriorly or posteriorly projecting microvilli depending on their orientation. They have a diameter of 0.15 μm , which may vary to a small degree. The afd microvilli, however, vary noticeably in length. The microvilli of the right afd vary randomly in length from 280 nm to 4280 nm and have a total combined length of about 227,000 nm. The microvilli of the left afd vary in length from 210 nm to 3500 nm, and have a total combined length of about 179,000 nm. The difference between the combined lengths of the left and right afd microvilli is about 48,000 nm (21%). Since the surface area of a cylinder is $\pi d \times \text{length}$ and the majority of the microvilli are of somewhat uniform diameter, 21% should be a close estimate of the difference between the surface areas created by the left and right afd microvilli. This might be an important determination of variation of afd sensitivity to stimuli.

The actual number of anteriorly projecting microvilli of the left reconstructed afd (51) is different from that of the right reconstructed afd (61). In addition, the afd microvilli are non-uniform, with respect to their length and overall shape, both within and between the two afd's. For purposes of illustration examine Figures 4-7; the four different views of the right afd. The actual number of posteriorly projecting microvilli is the same for both the left and right reconstructed afd's (47), however, notice that the length and the origins of these microvilli of the left afd are different from those of the right afd (Fig.'s 4-11).

The afd microvilli often branch from one original projection, near the dendrite enlargement, into two or more microvilli (Figures 4-11). The branching of the lateral projections is quite variant for both the left and the right afd's. Some lateral projections branch into more than five microvilli (e.g. Fig. 4a), while others do not branch at all. There seems to be no correlation between the amount of branching and the size or length of the lateral projections. Also, there is no uniformity to where the branch points occur. The majority of the branch points, however, occur within a certain distance from the dendrite enlargement. Notice that with one or two exceptions, all of the lateral projections, which

produce anteriorly projecting microvilli, have branched completely by the level of the anterior tip of the cilium. In some cases, many microvilli originate from a single lateral projection (e.g. Fig. 10c).

From the points where the projections branch into microvilli, the majority of the microvilli travel consistently parallel to the long axis of the cilium without changing their course of direction. The direction in which the microvilli travel is not strictly related to the origin of a microvillus relative to the dendrite enlargement. Some microvilli originate from the anterior of the dendrite enlargement and then travel posteriorly (e.g. Fig. 's 5d, e), and some microvilli originate from the posterior of the dendrite enlargement and then travel anteriorly (e.g. Fig. 's 6f, g). A general relationship, however, between the direction that the microvilli travel and the position of their origin can be stated: the majority of the microvilli that originate from the anterior 50% of the afd travel anteriorly, and the majority of the microvilli that originate from the posterior 50% of the afd travel posteriorly. Approximately 20% of the microvilli of the right afd, and 10% of the microvilli of the left afd, do not obey this relationship.

The distance that the microvilli travel from the dendrite enlargement, before they appear to be committed to traveling either anteriorly or posteriorly, is variable. Some of the microvilli appear to be guided anteriorly or posteriorly from the moment they originate from the dendrite enlargement, and others travel laterally, perpendicular to the long axis of the cilium, for a relatively large distance (up to 1700 nm) before bending to travel anteriorly or posteriorly. A small number of microvilli (1%) appear to be committed to traveling in one direction, but then bend 180° to reverse their direction of travel. There seems to be a zone along the Y-axis of the structure where the final direction of a microvillus is unpredictable, but anterior or posterior to this zone the course of a microvillus appears to be committed, anteriorly or posteriorly, and parallel to the body axis.

Chapter 3

STATISTICAL ANALYSIS OF VARIATION IN AFD STRUCTURE

In the previous chapter I described two afd's that I had completely reconstructed. Reconstruction helps to provide some insight into the morphology of the afd microvilli, and it is evident from Figures 4-11 that this morphology is non-uniform, within or between afd's, with respect to the length, the shape, and the occasional apparent misguided direction of the microvilli. The unique origin, path, and length of each microvillus is an interesting aspect of the afd's, however, it leads to sampling difficulties when comparing transverse sections among afd dendrites. A complete reconstruction of numerous afd's, therefore, would be necessary to accurately reveal the amount of variation between the two afd's of the same nematode, and among like-sided afd's from numerous isogenic nematodes. It is unreasonable, however, to attempt such a time-consuming analysis without the use of an elaborate computer-aided reconstruction program.

To circumvent this problem, and still obtain information on the microvillar variation, I identified several specific landmarks of the cilium that one may easily recognize in transverse sections through afd's. I used these landmarks to define several fixed reference planes, from which I compared sampled counts of microvilli between afd's from the same nematode and among afd's of numerous isogenic nematodes. A sampled count is the measure of the number of microvilli from a particular "sampled" transverse section through an afd. I employed several analysis of variance (anova) procedures to measure the amount of variation among sampled counts from numerous afd's, and from these I was able to estimate the amount of variation in the number of microvilli among those afd's. In addition, I used the anova's to test any experimental or inherent variables that may affect the number of microvilli counted. Using this approach I have examined the afd microvilli from 83 afd's.

Throughout this thesis I will be discussing the amount of variation among afd's. Unless otherwise stated, I will be referring to the variation among the number of the dendrite microvilli, as estimated from the sampled counts. In this chapter I will analyze some obvious experimental treatments that could have contributed to the observed variation of counts. Once these factors are considered, and I measure the contribution that they make to the total variation, I will be able determine whether there is morphological variation among afd's.

I will divide the contents of this chapter into four sections. 1) I determine which of the anterior reference planes produces the most accurate estimate of the anteriorly projecting microvilli, and which of the posterior reference planes produces the most accurate estimate of the posteriorly projecting microvilli. In addition, I will determine the amount of variation that is due to sampling difficulties. 2) I determine the effect of several treatments on sampled counts. These treatments are: (a) performing several replicate experiments to increase the sample size, (b) culturing conditions of illumination or darkness, and (c) left-sided versus right-sided afd's. 3) I examine the relationship between the number of anteriorly and posteriorly projecting microvilli. 4) with the above factors considered, I explore the contribution of variation among afd's which may be due to developmental noise.

Materials and Methods:

Counting Method

I obtained counts of microvilli from transverse sections by affixing a transparency film to each electronmicrograph and marking each microvillus with a fine point permanent colored pen. By this technique I protected the integrity of the original electronmicrographs and guarded against repeatedly counting individual microvilli. I subsequently repeated the counts of each transverse section at least three times to check the accuracy. For each afd, I counted as many transverse sections as could be obtained and designated the highest count of each region to represent the sampled count for that particular region.

Reference Planes

1. Anteriorly Projecting Microvilli

I designated several distinct landmarks of the afd cilium as fixed reference planes, and will refer to them as “regions” of the cilium because each includes several transverse sections. The factors that I considered when choosing the regions were: (1) each region must be well defined, and (2) each must be easily recognizable in the transverse sections. Fig.’s 12 -16 illustrate the different regions of the cilium in transverse sections through an afd. The landmarks that I have chosen arise from the different morphological characteristics of the cilium. Fig.’s 12A, B are serial transverse sections that reveal the emergence of the anterior tip of the cilium as one proceeds posteriorly through the sections. This distinct area of the cilium represents region one, and is delimited to a span of three transverse sections (200 nm). Region two is not as rigidly defined as region one, but it is easily recognizable, in the transverse sections, as the main “stalk” of the cilium between regions one and three (Fig. 13A). The span of region two is dependent on the length of the cilium, therefore, it is the only region whose span would vary among afd’s.

Fig.'s 12 A, B. Transverse sections through an afd of a wild-type *C. elegans* which reveal the characteristics of region one. A: Note the amphidial channel (a), the afd microvilli (m), and the emerging afd cilium (C). B: note the emerging afd cilium (C).

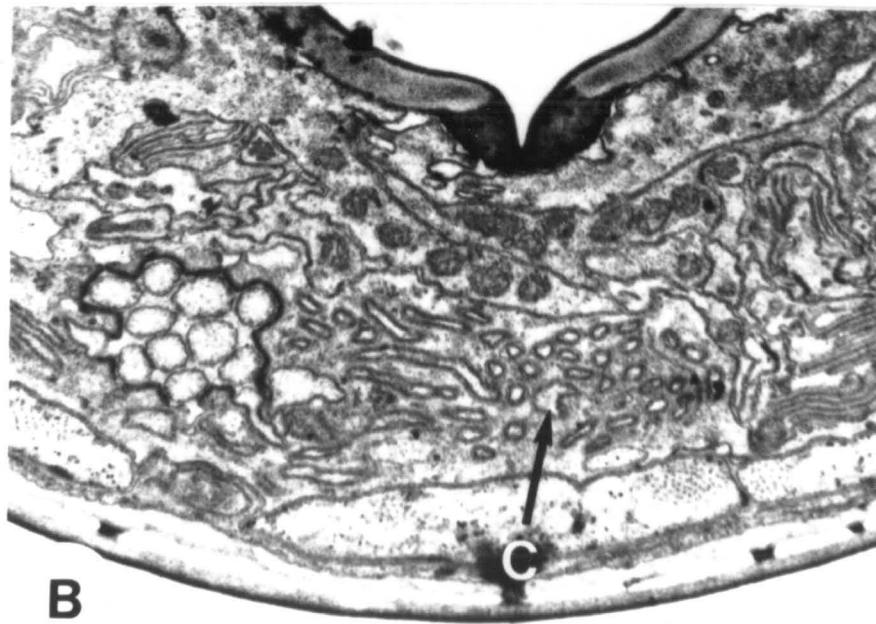
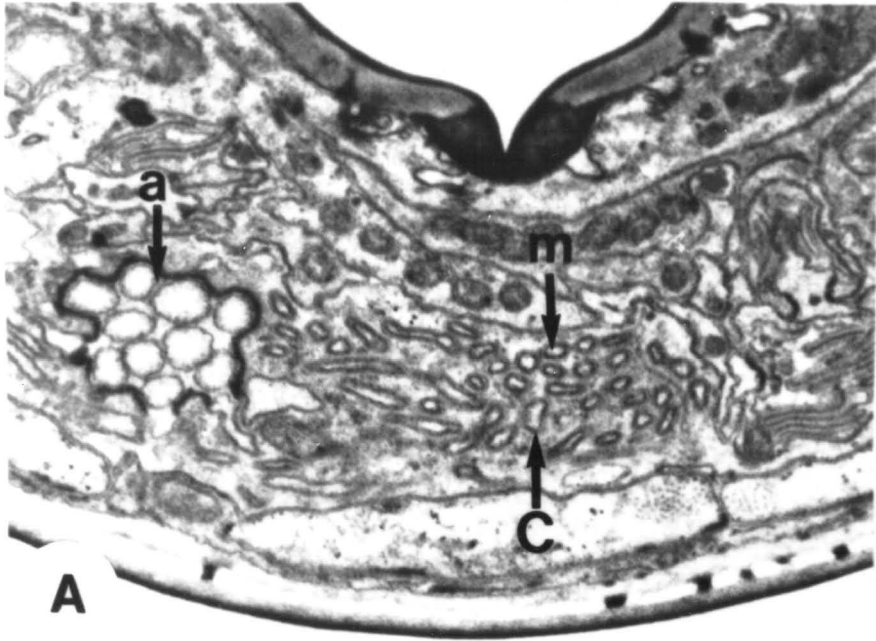
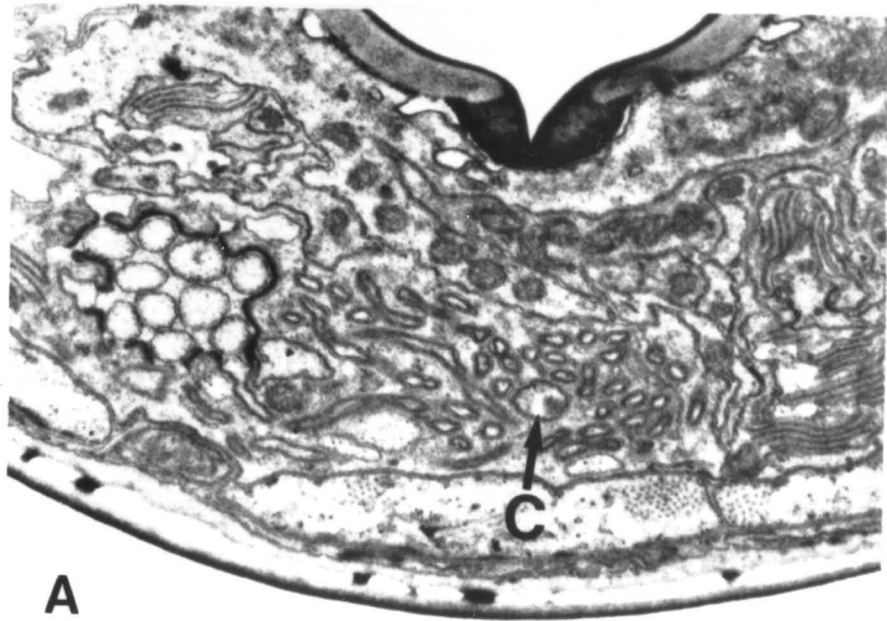
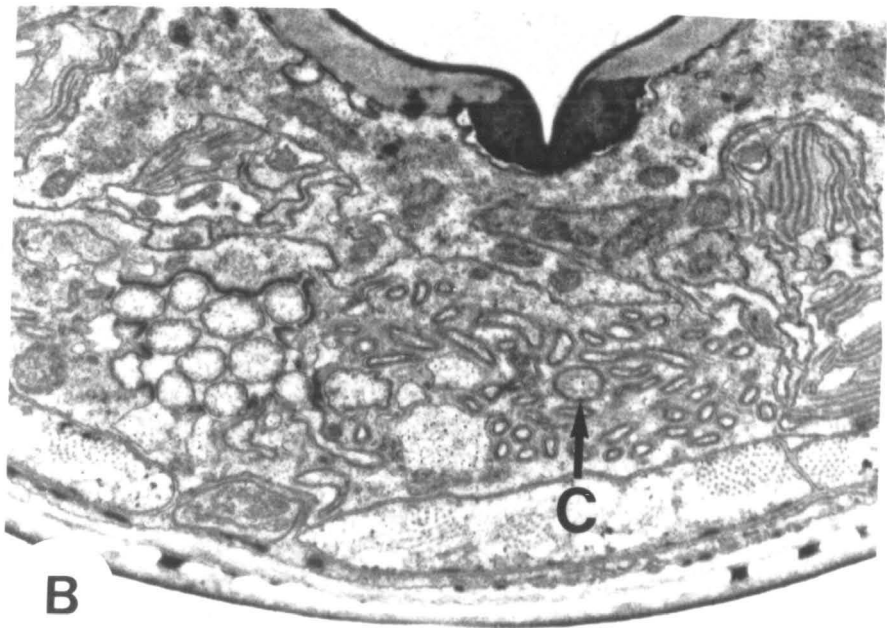


Fig.'s 13 A, B. Transverse sections through the anterior tip of a wild-type *C. elegans* which reveal the characteristics of regions two and three. A: transverse section through the main stalk of the afd cilium (C) which characterizes region two. B: transverse section through the transition zone of the afd cilium (C) which characterizes region three



A



B

The greatest span of region two was 16 transverse sections, and the least span was six transverse sections (of 65 nm each). Region three comprises the span of the cilium known as the transition zone (e.g. Perkins et al., 1986). It generally contains nine doublet-microtubules, a variable number of singlet-microtubules, and is easily recognizable in the transverse sections by the central cylinder to which the singlet-microtubules are attached (Fig. 13B). Like region one it is delimited to a span of approximately three transverse sections (200 nm). Region three is also situated very close to the dendrite enlargement, therefore, any short microvilli that may not be present in the transverse sections through regions one or two, will be counted in region three. The emergence of the dendrite enlargement characterizes region four, which is the final identifiable region associated with the anteriorly projecting microvilli (Fig. 14A). Immediately posterior to region four the dendrite cross-section becomes greatly enlarged, and the number of microvilli decreases rapidly as the transverse serial sections proceed posteriorly beyond the origins of the anteriorly projecting microvilli.

2. Posteriorly Projecting Microvilli

The lack of a posteriorly projecting afd cilium necessitates utilizing other means of characterizing the posterior reference planes. Proceeding posteriorly, Fig. 's 15A, B and Fig. 16 illustrate the disappearance of the dendrite enlargement from the transverse sections, which characterizes region five. This region is easily identifiable from serial sections, and it can be delimited to a span of approximately five sections. The only region that is not characterized by a specific landmark is region six. In transverse serial sections posterior to region five, the microvilli occasionally tend to increase in number to a certain maximum value. This increase in the number of microvilli, as one proceeds posteriorly past region five, may be explained by the fact that some of the microvilli branch near the dendrite enlargement from one original microvillus into two or more microvilli.

Fig.'s 14 A, B. Transverse sections through the anterior tip of a wild-type *C. elegans*. A: transverse section through the base of the cilium which characterizes region four. Note the appearance of the dendrite enlargement (DE). B: transverse section through the dendrite enlargement (DE)

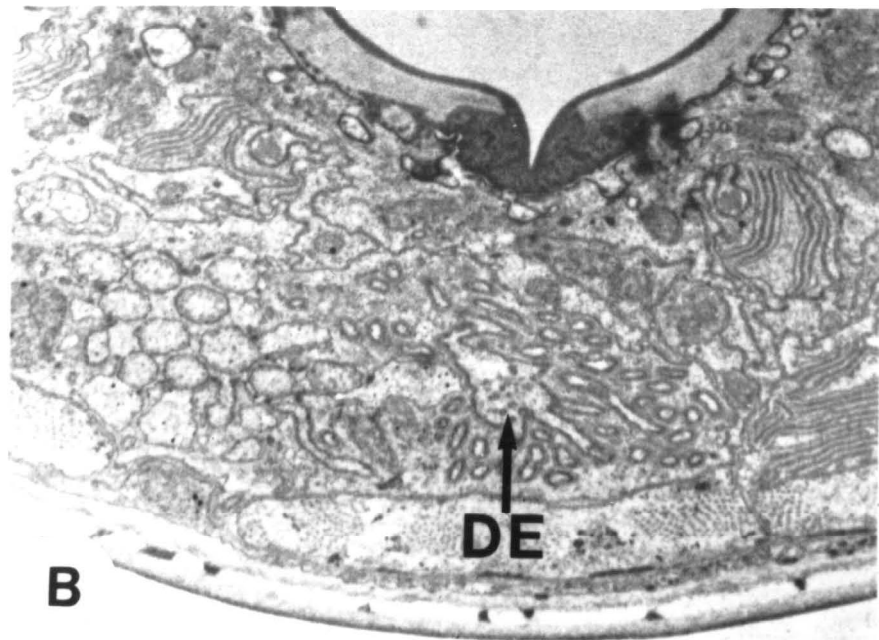
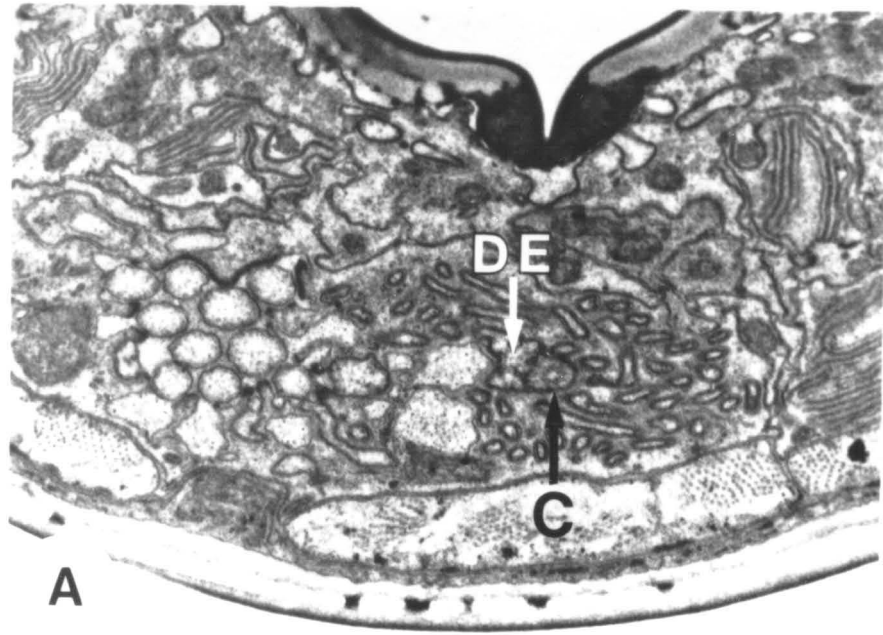


Fig.'s 15 A, B. Transverse sections through the anterior tip of a wild-type *C. elegans* which reveal the initial stages of the disappearance of the dendrite enlargement (DE), and characterize the beginning of region five. Note the dendritic process (DP) which transmits the signals from the dendrite to the nerve ring

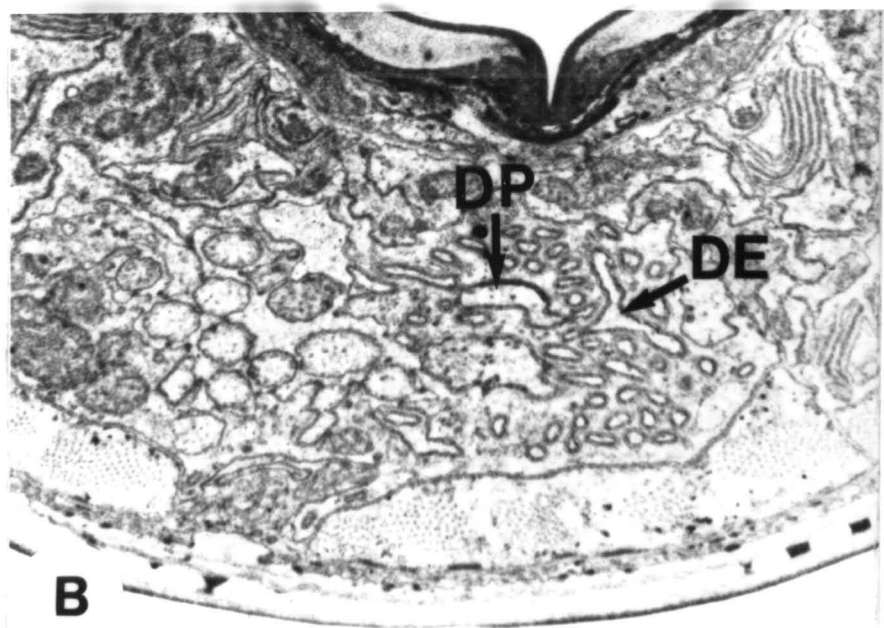
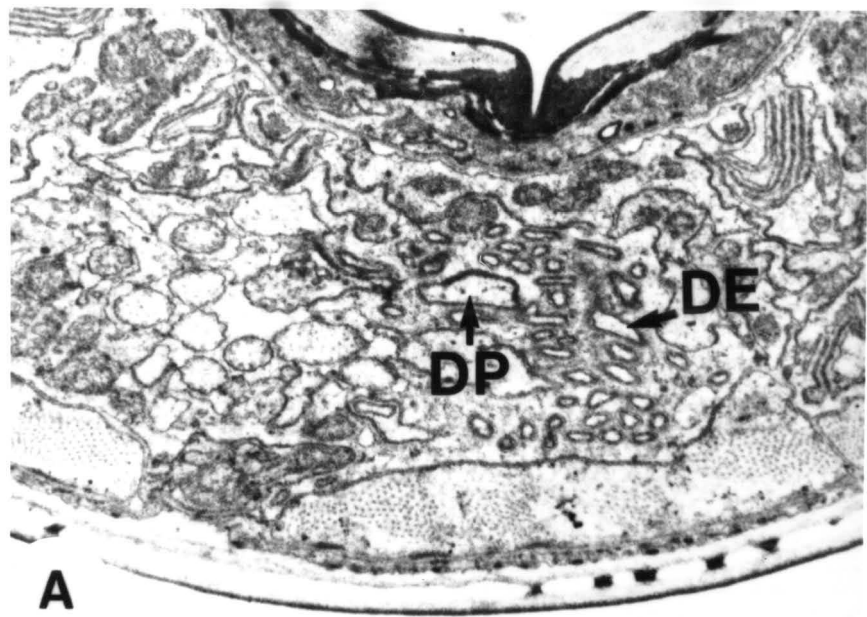


Fig. 16. Transverse section through the anterior tip of a wild-type *C. elegans* which reveals the characteristics of region five. Note the dendritic process (DP), and the disappearance of the dendrite enlargement (DE) which characterizes region five.



I have designated as region six the transverse section(s) posterior to region five that contain the maximum number of microvilli. Posterior to region six, the microvilli decrease in number as the shorter microvilli disappear from the transverse sections.

Data Analysis

Testing the equality of means between two populations that are affected by only one variable may be accomplished by performing a simple *t* test. When the equality of means, however, is to be tested between more than two populations and/or they are affected by two or more variables (or factors), analysis of variance (anova) procedures must be employed. The basic idea behind the anova is that the amount of variation among the combined individual measurements of all the groups tested (variation among measurements), is compared to the amount of variation among the means of the groups (variation among groups). If the variation among groups is significantly large with respect to the variation among measurements then one may conclude that the populations of the groups are significantly different from one another. On the other hand, if the variation among groups is nearly equal to or smaller than the variation among measurements, then one is unable to conclude that there is a significant difference among the groups. By taking into account the degrees of freedom, a probability value may be assigned to the variation.

The rejection of a null hypothesis when it is in fact true is called a Type 1 Error, and the probability of committing a Type 1 Error is equal to the chosen criterion significance level. On the other hand, the acceptance of a null hypothesis when it is in fact false is a Type 2 Error. Generally, one cannot specify the probability of committing a Type 2 Error, and the only way to reduce this error is to increase the sample size of the test (reviewed by Zar, 1984). I chose a significance level of 0.05, which by convention is usually considered to be an acceptable chance of committing a Type 1 Error, while not being so small as to result in an excessive chance of committing a Type 2 Error.

I performed several anova procedures with a split-plot design to test the equality of the means of sampled counts. Fig. 17 illustrates an example of a one-way or single-factor anova with a split-plot design. Here, the sampled counts were taken only from region three and the effect of location (left versus right afd's) is calculated from the differences between averaged counts within region three of left and right afd's of the same worm. These differences eliminate the illumination and worm effects, and therefore the location effect is estimated with greater precision. The estimated illumination effect, on the other hand, is subject to variability due to both locations and worms. This example illustrates one of the main applications of split-plot designs: the estimation of components of variability due to two or more sources.

All anova's were performed on SPARC-system hardware that utilized SAS-system software (version 6.0).

Fig. 17. Diagrammatic example of a split-plot design analysis of variance

Light ^{*a*}

Worm 1 ^{<i>b</i>}	
left ^{<i>c</i>}	right ^{<i>c</i>}

Worm 2	
left	right

Dark

Worm 3	
left	right

Worm 4	
left	right

^{*a*} Illumination = split-plots.

^{*b*} Worms = whole plots.

^{*c*} location (left/right) = subplot factor.

Section 1

Regional Analysis

Differences among Regions

The best region from which to obtain sampled counts is preferably the one that produces the most accurate estimate of the actual number of microvilli of that afd, while exhibiting the least amount of variation in the number of microvilli among the transverse sections that span the region. This was determined by analyzing counts in all transverse sections of the reconstructed afd's as well as counts sampled within the different regions of many afd's. From sampled counts, I determined whether there were significant differences among the anterior regions of numerous afd's. This helps to characterize the afd for future study, but more importantly it addresses the issue of whether microvillar data should be obtained and analyzed according to the specified regions. If the sampled counts from the various regions are not significantly different from one another, then identification of regions for the purpose of comparing sampled counts among afd's would not be necessary, and comparing sampled counts among afd's would be easier and less time consuming. If, however, the sampled counts from the different regions are significantly different from one another, then for greatest accuracy it would be necessary to compare the sampled counts from the most representative region. This was determined by comparing, in the two reconstructed afd's, the mean count in each region with the actual number of microvilli.

Each region of an afd dendrite spans several transverse sections, each of which may produce a different sampled count of microvilli. The variation within regions is the potential variation in the number of microvilli among the sections through a region of a given afd dendrite. By completely reconstructing two afd's, I have revealed that the variation within regions is a direct result of the complex morphology of the dendrite

microvilli. The region with the least variation was determined from both the reconstructed afd's and from the larger set of sampled cells. From the two completely reconstructed afd's it was possible to obtain the actual variation among all sections through each region. To compare the microvilli of many afd's, however, I had to rely on a transverse section sampled from each region.

For the 83 afd's that I have sampled, the total variation among the sampled counts may be due to: (1) differences in the actual number of microvilli among afd's; (2) differences in the morphology of microvilli among afd's which affect whether or not it passes through a region; (3) variation within the regions of each afd. Since the sampled afd's are from isogenic worms, any differences in either the actual number of microvilli or the morphology of their dendrites may be due to developmental noise. The variation within regions, however, is obviously a result of sampling difficulties and must be taken into account prior to estimating the real degree of variation which results from developmental noise.

From the two reconstructed afd's one may determine the exact amount of variation within regions for each of the six regions and the contribution that variation within regions contributes to the total variation between these afd's. Analysis of the left and right reconstructed afd's from one nematode may have revealed numerous differences in their microvillar morphology, however, I did not rule out the possibility that the afd's from one side of isogenic worms may differ in morphology among worms. Therefore, in the comparisons of sampled counts among numerous afd's, I analyzed the variation among like-sided afd's and tested for a significant difference between sides.

I analyzed each of the six regions from the two afd's that I completely reconstructed, determined the span of each region from the number of transverse sections, and counted the microvilli in each section. From these, I calculated the mean of the sampled counts for each region and the variation among the sampled counts within each region (standard deviation). I then compared the sampled count means to the actual

number of microvilli and determined the accuracy of estimating the actual number of microvilli from the sampled counts.

To determine whether significant differences could be detected among the sampled counts of the anterior regions of many cells I performed a single-factor anova (split-plot design) on 109 sampled counts from left afd's and 110 sampled counts from right afd's. After performing an anova I applied multiple comparisons tests, using the Bonferroni comparisons method (e.g. Mason et al., 1989a), on each of the anterior regions. As I had not yet determined whether the sampled counts from the left and right afd's could be pooled to increase the sample size the sampled counts from the left and right afd's were analyzed separately. Sampled counts of the posterior regions were not analyzed, as the sampled count that most accurately represents the posteriorly projecting microvilli was determined to be the highest count from either region five or six (see below).

The mean numbers of microvilli from each region of the two reconstructed afd's, along with the actual number of anteriorly and posteriorly projecting microvilli, are illustrated in Tables 1 and 2. The actual number of microvilli includes all processes greater than 200 nm in length. For purposes of illustration, examine the data for the anteriorly projecting microvilli of the left reconstructed afd (Table 1, pg.28). The actual number of microvilli is 51, and the mean number of microvilli from the four transverse sections that pass through region three is 37. The difference is 14 microvilli. This difference is the "sampling error", which is the absolute error in estimating the actual number of microvilli from sampled counts of microvilli. The sampled count of the left afd provides an estimate of the actual number of microvilli that is 27% lower than the true value. For the right afd, the mean number of microvilli counted in sections through region three is 46, which is 15 microvilli or 25% lower than the true value of 61. The sampling errors of regions two and three are slightly lower than those of regions one or four.

Table 2. Numbers of microvilli in the posterior regions of the left and right reconstructed afd's

a. Left afd: actual number of posteriorly projecting microvilli = 47

region	t. s. ^a	mean ^b	std. dev.	mean - actual ^c	sampling error ^d
5	5	33	2.0	-14	-30%
6	3	30	0.6	-17	-36%
max. value ^e					
5 + 6	7	35		-12	-26%

b. Right afd: actual number of posteriorly projecting microvilli = 47

region	t. s.	mean	std. dev.	mean - actual	sampling error
5	5	29	0.8	-18	-38%
6	3	33	0	-14	-30%
max. value					
5 + 6	7	33		-14	-30%

^a number of transverse sections through a region.

^b mean number of microvilli per region.

^c the mean number of microvilli of that region minus the actual number of posteriorly projecting microvilli.

^d sampling error of that region = [(mean - actual number) / actual number of posteriorly projecting microvilli].

^e the maximum observed value of all sampled counts within region's five or six.

There is a directly proportional relationship between the sampled microvillar counts from the anterior regions of an afd and the actual number of anterior microvilli that the afd has. The mean numbers of microvilli counted in each of the anterior regions of the left afd are 83%, on average, of the mean numbers of microvilli in each of the corresponding anterior regions of the right afd. These estimates reflect closely the degree by which the actual numbers differ. Therefore, for the 83 afd's that I sampled, the differences among the sampled counts from region three probably reflects, proportionally, the differences among the actual numbers of their microvilli.

The actual number of posteriorly projecting microvilli of the left and right reconstructed afd's of this worm turned out to be identical (Table's 2a, b). However, the mean numbers of microvilli in sections through regions five or six of the left afd differ by 10% from those of the corresponding regions of the right afd. One factor, which may contribute to this difference is the absence of well-defined planes of reference identifying the posterior regions. For the two reconstructed afd's, the highest available count of the posteriorly projecting microvilli, from either region five or region six, is 35 for the left afd and 33 for the right afd. These values are similar, and the estimates that they produce of the actual number of microvilli of each afd are a better reflection of the (zero) difference between sides. Therefore, for the analysis of anteriorly versus posteriorly projecting microvilli (Section 3), I have estimated the number of the posteriorly projecting microvilli as the highest sampled counts that I obtained from regions five or six.

For the 83 afd's that were sampled, single-factor anova indicated that the effect of region is significant for right afd's ($p = 0.006$) and nearly significant for left afd's ($p = 0.06$). The LSmeans for each of the anterior regions of the left and right afd's are given in Table 3. These means were a part of the SAS anova output and were estimated by least squares fitting of the anova model to the data (e.g. Mason et al., 1989b)

Table 3. Multiple comparisons of the least squares means for the anterior regions of the left and right sampled afd's of wild-type *C. elegans*.

a. Least squares means of each of the anterior regions of the left and right sampled afd's

Left afd's				Right afd's			
region	LSmean ^a	std. dev.	d.f.	region	LSmean	std. dev.	d.f.
1	37.8	9.2	13	1	37.1	10.7	13
2	42.0	6.4	24	2	42.1	6.6	25
3	42.7	7.0	39	3	42.0	7.8	42
4	38.7	7.1	29	4	42.0	7.1	26

b. Multiple comparisons of the least squares means for the anterior regions of the left and right sampled afd's

Comparison	d.f.	p ^b	m ^c	significant
Between regions:				
1 vs. 2 (left)	37	0.056	3	
1 vs. 2 (right)	38	0.02	3	*
1 vs. 3 (left)	52	0.01	2	*
1 vs. 3 (right)	55	0.01	2	*
1 vs. 4 (left)	42	0.65		
1 vs. 4 (right)	39	0.01	2	*
3 vs. 4 (left)	68	0.01	2	*
3 vs. 4 (right)	68	0.93		

^a mean estimated by least squares fitting of the anova model to the data.

^b Significance level.

^c Bonferroni correction factor = number of pairs of means.

An investigation of each region was performed by the method of multiple comparisons with Bonferroni correction. This revealed significant differences between the mean counts of regions one and three for both the left and right afd's (Table 3). Significant differences were also detected between the mean counts of regions one and four of the right afd's and regions three and four of the left afd's. Counts sampled from either region one or region four are significantly lower estimates of the actual number of anteriorly projecting microvilli than those obtained from region two or region three. The lower sampled counts from region one may be explained by the fact that some of the shorter microvilli do not extend to region one, located at the apex of the cilium. The lower sampled counts from region four may be explained by the fact that some of the transverse sections do not include the microvilli that originate from the apex of the dendrite enlargement or that have originated more posteriorly but have not branched by this region. These results indicate that sampling from regions two or three would provide better estimates of the actual number than sampling randomly from any of the other regions.

These results are in agreement with my findings from the analysis of counts from the two reconstructed afd's. These provide strong statistical and quantitative arguments for choosing regions two or three for obtaining the best estimates of the actual number of anteriorly projecting microvilli and for choosing the highest count from regions five or six for obtaining the best estimates of the actual number of posteriorly projecting microvilli of an afd dendrite.

Variation within Regions

I used two approaches to determine the amount of variation within regions. From these, I determined whether variation within regions contributes significantly to the total variation among sampled counts.

Approach 1

The data from the two reconstructed afd's provides an exact measure of the amount of variation within regions for each of the six regions. From the differences among regions, I determined in the preceding section that the sampled counts from region three will provide the best estimate of the actual number of anteriorly projecting microvilli, and the highest sampled count from either region five or six will provide the best estimate of the actual number of posteriorly projecting microvilli. Here, I focus on the amount of variation within region three and within regions five and six of the two reconstructed afd's. These are accurately measured values on a sample of two afd's and, though a small sample, should provide one estimate of the variation within regions that one can expect to find in non-reconstructed "sampled" afd's. I analyzed the microvillar counts from the serial transverse sections of the left and right afd's that I had reconstructed, and calculated the mean number of microvilli and the variation about the means for each region (standard deviation). This variation among sampled counts within a region is the variation within regions.

Between two adjacent regions there is no clearly distinct plane of reference by which one may identify a transverse section as belonging exclusively to one region or the other. Therefore, for the two reconstructed afd's, the data from such transverse sections was used in the calculations of both adjacent regions, and I refer to this as regional overlapping. Since the regional determination of such transverse sections from sampled afd's is more difficult than the regional determination from transverse sections of reconstructed afd's, the regional overlapping of the reconstructed afd's provides a more conservative estimate of the variation within regions for the sampled afd's. This estimate of variation was calculated from the number of microvilli in sections through region three of the two reconstructed afd's (Table's 1a, b). For the left afd, the number of microvilli in the four transverse sections of region three vary with a standard deviation of 0.6, and for the right afd, the standard deviation is 2.0. The corresponding root mean square variation

for region three of the left and right afd's is 1.5. The variation within the posterior regions were in the same range (Table's 2a, b). The standard deviation of regions 2 + 3 combined is larger than either region two or three alone (Table 1). The number of anteriorly and posteriorly projecting microvilli do not appear to be correlated (see Section 3 results).

Approach 2

My second approach is based on calculations from sampled counts of numerous afd's and estimates the amount of variation within the combined span of two regions, region two and region three. I will refer to this as the within-afd variation to distinguish it from the variation within regions.

The combined span of regions two and three extends over a much larger domain of the dendrite than the span of region three alone. The morphological variation of the microvilli within this extended domain will be potentially larger than that within region three alone, therefore, the within-afd variation is a conservative estimate of the variation within the span of region three alone. The mean and variation in the number of microvilli from sampled counts within regions two and three are similar (Table 3), therefore, I can assume that the estimates of the within-afd variation from this approach will be close to, but slightly more conservative, than the measurements of the variation within regions that I obtained from region three alone.

I employed two separate methods of estimating the variation within the combined span of regions two and three. In the first method I performed an anova (split-plot design) on combined sampled counts from regions two and three of 74 afd's. The general linear model of the anova defines each of the variables that contribute to the total observed variation among sampled counts. It starts with a mean overall number of microvilli, which is regarded as a fixed background constant number of microvilli. Deviations from this mean are considered in the model to be due to two factors: (1) variation in the number of microvilli among afd's, and (2) variation within afd's, which in this case is the variation

within the span of regions two and three. The general linear model may be expressed as $Y_{ij} = \mu + A_i + E_{ij}$, where Y_{ij} is the j^{th} observed sampled count from regions two and three of afd # i , and μ is the fixed overall background number of microvilli. The A_i 's and E_{ij} 's are independent random variables, where the A_i 's express the deviations from μ that are due to random variation among the different afd's, and the E_{ij} 's express the deviations from μ that are due to the variation among counts from random sections through regions two and three.

In the second method I used a more direct approach to estimate the variation within region three. From 11 afd's of which three or more random sections within regions two and three were obtained, I directly calculated the mean and variance of the counts sampled from each afd without anova procedures. From these 11 variances I then calculated the root mean square variance of all 11 afd's.

These estimates of variation of counts sampled within regions two and three were intended to provide a more conservative estimate of the variation than that within region three alone. The within-afd variation of the 74 afd's is estimated as a standard deviation of 3.7. This is the square root of the MS_{Error} from the anova described above. For the 11 afd's which exhibited three or more counts sampled from within regions two and three combined of each afd, the root mean square standard deviation that I calculated was 3.1. Notice that the variation within these combined regions is indeed larger than that within region three alone of the left and right reconstructed afd's (Table 4).

These conservative estimates of the variation within region three will be used when I determine whether there are real differences among afd's (Section 4).

Table 4. Within-afd variation and variation within regions of sampled and reconstructed afd's

afd's	Within-afd variation of region 2 + 3 ^a	Variation within region 3 ^b
Sampled:		
74 afd's ^c	3.7	
11 afd's ^d	3.1	
From reconstruction:		
Left afd	1.5	0.6
Right afd	2.6	2.0

^a the standard deviation of sampled counts from within the span of region two + region three.

^b the standard deviation of the sampled counts from region three of the two reconstructed afd's.

^c the 74 afd's which each produced two counts (one from region two and one from region three) of sampled afd's.

^d the 11 afd's which each produced three or more counts from within regions two and three of sampled afd's.

Section 2

Analysis of Experimental Variables

To obtain a large sample size I performed five replicate experiments over the period of one year. Although the conditions of the experiments were maintained the same I did not rule out the possibility that unknown differences among the experiments may have affected sensillum morphology. To determine whether illumination during culture has an effect on the number of microvilli, in each of five experiments I exposed two groups of isogenic nematodes to the conditions of either continuous illumination or continuous darkness. In addition, I wanted to determine whether sampled counts from left afd's are significantly different from those of the right afd's. For this comparison the counts were grouped so as to compare all left counts to all right counts regardless of whether the left and right afd's were "paired". I performed a single-factor anova (split-plot design) on the counts sampled from region three to determine: (1) if there were significant differences among the counts sampled from the five replicate experiments; (2) if there were significant differences between the counts sampled from the light and dark groups; (3) if there were significant differences between counts sampled from left and right afd's. The design was similar to that of the example that I described earlier (Fig. 17). The only difference is that in this case the additional treatment of replicate experiments is added to the test.

No significant difference was detected among the five replicate experiments, between the light and dark exposed afd's, or between the left and right afd's (Table 5).

Table 5. Analysis of variance of counts sampled from region three

Treatments:	d.f.	significance level
Five replicate experiments	4	0.25
Illumination	1	0.51
Location (left vs. right)	1	0.46

In addition, I performed a linear correlation analysis to determine if there is a relationship between the size of sampled counts from left and right afd's. I detected a positive correlation between the sizes of sampled counts of the left and right afd's ($r = 0.55$, d.f. = 33, significance level = 0.05). Thus, a worm with a larger number of microvilli on one side is likely to have a larger number of microvilli on the other side.

Section 3

Anterior Versus Posterior Projections

The anteriorly and posteriorly projecting microvilli are two variables of an afd that may be intimately associated with one another. They both originate from the same afd enlargement, thus any changes in the physiology of the dendrite that affects one probably affects the other. In addition, the precision of developmental control is most likely the same for the anteriorly and posteriorly projecting microvilli of any one afd. For this reason, if the numbers of anteriorly and posteriorly projecting microvilli are correlated, it may be justified to state that their numbers are dependent on one another or on a third phenomenon. In this section I focus on the relationship between the anteriorly and posteriorly projecting microvilli, and I determine whether their numbers differ significantly from one another.

Results

For the two reconstructed afd's, the difference between the actual number of anteriorly projecting microvilli is 10 microvilli (Table's 1a, b). Since the actual number of posteriorly projecting microvilli of the left and right afd's are the same, this suggests that there may be no relationship between the anteriorly and posteriorly projecting microvilli, however, a sample size of two is too small. There were 9 afd's for which the numbers of both the anteriorly and posteriorly projecting microvilli could be counted. For these the numbers of microvilli projecting anteriorly and posteriorly were not significantly correlated ($r = 0.194$, d.f. = 7).

Section 4

Variation Among Afd Microvilli

Any variation among microvillar counts, above and beyond that explained by the variation within the sampled region, is due to morphological variation among afd's. Such variation may be between the left and right "paired" afd's of a worm (within-worm variation), or among like-sided afd's from numerous isogenic worms (among-worm variation). In this section I focus on the contributions that the within-worm and among-worm variations make to the total variation among afd's.

Methods

Counts were from one section sampled in region three of each afd. I employed a single-factor anova (split-plot design) on counts from 38 afd's in 19 isogenic worms (19 left afd's, 19 right afd's) to determine the amount of within-worm variation. In addition, I estimated the amount of among-worm variation, from 83 afd's as described below. The 83 afd's included the above 38 left and right afd's in 19 worms, and another 45 afd's (from either the left or the right afd) of another 45 worms. The anova model assumed was the same as the general linear model that I used in section 1 to determine the within-cell variation. In this case, however, only one section came from the sample region and the two random variables I assume to be responsible for variation among afd's are within-worm differences and among-worm differences instead of within-region differences and among-worm differences. The model $Y_{ij} = \mu + A_i + E_{ij}$ represents the sampled count (Y_{ij}) from region three of worm # i at side # j . The sampled count is once again assumed to consist of deviations from a fixed overall background constant number of microvilli (μ). However, in this case Y_{ij} may be affected by the independent random variables A_i (among-worm variation) and E_{ij} (within-worm variation). The A_i effects reflect any

random variation among worms which affect the sampled count Y_{ij} , and the E_{ij} 's express the random variation within worms (left versus right) which affect the count.

The single-factor anova with random effects directly produces an estimate of the within-worm variation, while the among-worm variation may be calculated from the least squares fit equation provided in the computer output. This equation takes into account the relationship between the experimental design, the amount of available data, and any missing values.

Results

A significant difference was detected among the counts sampled from like-sided afd's (among-worm variation: $p = 0.05$, d.f. = 82) thus, the variation among worms is significant. The among-worm variance, the within-worm variance, and the conservative estimates of the variance within region three are listed in Table 6. The among-worm variance (24.22) was calculated from the least squares fit equation, and an estimate of the within-worm variance (30.94) was indicated by the MSError of the anova. I performed an F-test to determine whether the variances among- and within-worms were equal with the result: the contributions that the within-worm and among-worm variation make to the total variation are not significantly different ($F = 1.28$, $p > 0.5$).

In addition, I performed F-tests to determine whether the conservative estimates of the variance within region three are equal to either the within-worm variance or the among-worm variance (Table 6). These tests helped me to determine whether there were real differences among- or within-worms or instead, whether the differences could be attributed to variation due to sampling within region three. Significant differences were detected between the variance within region three and both the among-worm and within-worm variances.

Table 6. F-tests for the comparisons of the within-afd variance to the within- and among-worm variances.

sample	abbreviation	variance	d.f.
within-worm variance	W/W	30.94	18
among-worm variance	A/W	24.22	82
within-afd variance, 74 afd's	WAV ₇₄	13.69	73
within-afd variance, 11 afd's	WAV ₁₁	9.30	10

calculated F-value	significance level	significant
F: W/W / WAV ₇₄ = 2.26	<0.05	*
F: W/W / WAV ₁₁ = 3.33	<0.01	*
F: A/W / WAV ₇₄ = 1.77	<0.05	*
F: A/W / WAV ₁₁ = 2.60	<0.01	*

The value of the variance within region three was significantly lower than either the within-worm variance or the among-worm variance, thus, the noticed variation within- and among-worms is not entirely due to variance within region three but that there are real differences among afd's. These differences are due to real differences within worms (between sides), and real differences among isogenic worms (like-sided afd's).

Chapter 4

SUMMARY AND CONCLUSIONS

The main focus of this thesis is to examine the morphology of afd's from wild-type *C. elegans* and to determine the degree to which the afd's vary in their morphology. The microvilli of the two afd's that I reconstructed are much less organized and non-uniform than indicated by the previous computer reconstruction by Ward et al. (1975). In their reconstruction of the afd all of the microvilli appear to originate directly from either the anterior or posterior surfaces of the afd, but in my reconstructions I found that the majority of the microvilli originate from processes which project laterally from the afd's. I also noticed that microvilli vary within and between afd cells with respect to their length and overall shape. For example, the microvilli of the right afd vary in length from 280 nm to 4280 nm. The variation in the length of the afd microvilli is somewhat unique, as the majority of other types of microvilli such as vertebrate brush border microvilli and invertebrate photoreceptive microvilli are quite uniform in length. The length of other types of microvilli may be 1 μm in squid (Saibil and Hewat, 1987) and 12 μm in snails (Brandenburger et al., 1976), but within a species the length of the microvilli is normally quite constant. Another obvious difference between the afd microvilli and vertebrate brush border microvilli or invertebrate photoreceptive microvilli is that the afd microvilli are not as neatly organized, and the afd microvilli often branch from one original projection, near the dendrite enlargement, into two or more microvilli. The positions where the branching takes place is non-uniform and provides one explanation for the noticeable variation in the length of the microvilli. The difference in the summed lengths of the microvilli between the two afd' is approximately 21%. Since the diameters of the microvilli are quite uniform, this corresponds to a difference of approximately 21% in the surface area of the two afd's. One of the constant factors of the afd microvilli, however, is the post-branching direction of travel of the microvilli. Once the microvilli have branched, they travel either strictly

anteriorly or posteriorly. A small percentage of the microvilli may travel slightly obliquely, but the vast majority of the microvilli travel parallel to the afd cilium. In addition, a small percentage of the microvilli that originate from the anterior of the dendrite enlargement travel posteriorly, and from the posterior of the dendrite enlargement travel anteriorly.

The cilia of the two reconstructed afd's exhibited similar diameters, however, the lengths of the cilia differ by approximately 21%. For the two reconstructed afd's there appears to be an inverse relationship between the length of the cilia and the number of microvilli: the left afd has 21% less microvilli and a 21% longer cilium than the right afd. However, a linear correlation analysis of 14 afd's indicates that there is no significant relationship between cilium length and the number of microvilli ($r = 0.072$, d.f. = 12).

Counts from a larger number of afd's were sampled from specific defined regions. The anterior regions that produce the most accurate estimate of the actual number of anteriorly projecting microvilli are regions two and three. I collected sampled counts from region three because it was more easily identifiable in transverse sections and narrower than region two. For the two reconstructed afd's, the mean sampled counts from region three were lower than the actual numbers of anteriorly projecting microvilli. The actual number of anteriorly projecting microvilli was 51 for the left afd and 61 for the right afd, and the mean sampled counts from region three were 27% lower on the left and 25% on the right. Therefore, the sampled counts from these afd's nearly equally estimate the degree of the difference between the actual number of their microvilli and the sample was proportional to the actual number. For the posterior regions I found no suitable landmark by which to identify reference planes in the transverse sections. For this reason, the highest number of microvilli from approximately seven sections through the posterior regions was used to represent the actual number of posteriorly projecting microvilli. For the two reconstructed afd's, the highest number of microvilli from either of regions five or six was 35 for the left afd and 33 for the right afd. Since the actual numbers of posteriorly

projecting microvilli in this individual are the same (47), the sampling errors of the sampled counts are similar.

A small amount of variation is present among the sampled counts within region three of any one afd. This I call the regional variation and it is due to the complex morphology of the afd microvilli. Therefore, before determining whether there is variation among afd's, one must estimate the amount that regional variation contributes to the noticed variation among sampled counts of different afd's. From the two reconstructed afd's, the root mean squared standard deviation of the regional variation was 1.5. A conservative estimate of the within-afd variation (regional variation) from 74 afd's was 3.7, and from 11 afd's that each produced three or more sampled counts, the regional variation was 3.1. These were the estimates of regional variation that I used in my final analysis to determine whether there are real differences among the sampled counts from afd's.

Factors that I considered in an anova were: (1) the data was obtained from five replicate experiments; (2) some afd's were exposed to illuminated conditions and others were exposed to darkness; (3) the left and right afd's may exhibit different numbers of microvilli. Overall, no difference was detected among the sampled counts of the different experiments. This indicates that the afd were not significantly affected by any possible slight environmental differences among the experiments. I was unable to detect a significant difference between the illumination conditions. In addition, study of the transverse sections through either of the light and dark exposed afd's did not reveal the presence of vesicles or multilamellar bodies commonly associated with the microvillar degradation of invertebrate microvillar photoreceptors. These results indicate that if afd microvilli of *C. elegans* maintain a photoreceptive function, then they do not exhibit the microvillar degradation which is common to many invertebrate photoreceptive microvilli.

Sampled counts of the left and right microvilli were not significantly different. Furthermore, a linear correlation analysis revealed a positive correlation between the left

and right "paired" afd's of 19 worms. This indicates that if the sampled counts from the left afd are high then the sampled counts from the right afd are likely to be high. On the other hand the anteriorly and posteriorly projecting microvilli were not significantly correlated.

My analysis of 83 afd's revealed that the amount of within-worm variation between the left and right afd's (std. dev. = 5.6) is approximately equal to the amount of variation among the left sampled counts or right sampled counts of numerous isogenic worms (std. dev. = 4.9). By performing F-tests, I determined that conservative estimates of the regional variation are significantly smaller than either the within-worm variation or the among-afd variation. This indicates that these variations are not entirely due to regional variation among sampled counts taken from region three, which is in accordance with my observations of the two reconstructed afd's. One may obtain an idea of the degree of the true variation among afd's from the difference between the conservative estimates of the regional variance and the measurements of the within- and among-worm variances. These results indicate that the range of the true within-worm afd variation (standard deviation) may be on the order of 4.2 - 4.7 microvilli, and the range of the true among-worm afd variation (standard deviation) may be on the order of 3.2 - 3.9 microvilli. This is about 13% and 10% respectively, of the mean number of microvilli sampled from region three of the left reconstructed afd, and 10% and 8% respectively, of the mean number of microvilli sampled from region three of the right reconstructed afd.

Discussion

The nervous system is particularly suitable for the study of developmental noise because subtle structural phenotypic variation may be readily assessed. They may have more pronounced consequences than in many other organs, consequently one may expect their development to be more tightly controlled. The gross overall morphology of the

wild-type *C. elegans* nervous system is highly invariant among isogenic worms (Chalfie, 1984; Chalfie and Sulston, 1981; Ward et al., 1975; White et al., 1976). This observation, however, begins to break down at the exceedingly finer details of the morphology, where at some point the effects of developmental noise apparently become manifest. Some aspects of phenotypic variability among identifiable neuronal structures may include: (1) the number of neurons of which the structure is composed; (2) the relative position of these neurons within the structure; (3) the morphology of their axonal and dendritic processes; (4) the number and pattern of their synaptic connections (reviewed by Stent, 1981). White et al. (1983) describe synaptic variability among *C. elegans* in three isogenic worms, where the number of synapses varied between classes of neurons that were synaptically connected. White et al. (1976) previously described two types of variability: (1) synaptic connections, where there is some variability in the number of synapses that a given motor neuron receives, and; (2) morphological variability, where there is some variability in the relative positions of cells within the right ventral ganglion, in addition to variability of the branching patterns of some neurons. The worms used in their studies had the same genotypes and were grown up under identical conditions to mature adults. Hall and Russell (1990) serially reconstructed identified neurons in the posterior nervous system of *C. elegans* and studied the complete pattern of synaptic interactions. They found that about 80% - 90% of the synapses fell into repeated classes of synapses while the remaining synapses are widely scattered and irreproducible. The conclusion that they drew from this was that approximately 10% - 20% of these contacts may be developmental mistakes which reflect a degree of developmental noise. Sulston and Horvitz (1977) examined the postembryonic somatic cell lineages of *C. elegans* and determined that they are generally invariant, with a fixed pattern of cell divisions and a fixed developmental program defined for every daughter cell. They described, however, five basic types of variations in the details of this developmental program: (1) variation in the pattern of cell divisions of the intestinal nuclei and the hypodermal cells of the ventral cord; (2) variation

in the pattern of cell deaths, where the progeny of the right lateral neuroblast (Q2.pp), which normally dies, in one case survived into the adult; (3) variation in which of two alternative lineage programs a given cell will follow, where the fate of the majority of cells appears to be determined intrinsically according to its lineage history, however, some cells, like the ventral cord hypodermal blast cells P5.p and P6.p, have a fate that is determined extrinsically according to the position they assume in the ventral cord; (4) variation in the precise order of specific events, such as the timing of cell divisions and the order of migration and division where sometimes a cell will migrate and then divide or vice-versa; (5) variation in the precise positions of cell nuclei, where the development of both the ventral nerve cord and the somatic musculature leads to variable intercalation of new progeny cells. Further work by Sulston et al. (1980) showed developmental variability of the male copulatory apparatus. For example, the loss of one or more rays or the fusion of rays 8 and 9 are apparently common errors in the developmental process.

Variability among isogenic organisms has also been described for the sensory neuron projections in *Drosophila* (Ghysen, 1980), locust interneurons (Goodman, 1978; Goodman, 1974; Pearson and Goodman, 1979), the structure and development of neurons in *Daphnia magna* (Macagno et al., 1973), and phenotypic variation in major psychiatric disorders of identical twins (Mellon and Clark, 1990). Such variability among these isogenic individuals probably represents the effects of developmental noise, as the genetic component of variation is eliminated, however, can the effects of environment be ruled out in the case of the twins? It is possible that the genetic polymorphisms such as chromosome loss or somatic recombination occur during development. This would give rise to differences in the genetic constitution of the somatic cells, however, this in itself merely represents one source of developmental noise.

Oster et al. (1987) indicate that limb morphogenesis of the salamander *Ambystoma mexicanum* appears to be quite deterministic on a macroscopic scale, but on a microscopic scale the cellular activities during the formation of the limb appear to be nearly random.

Here, they suggest that order emerges only as an average outcome of numerous individual motions, each of which has a large stochastic component, and they hypothesize that much of the development is constrained by the probability of morphogenic events occurring under certain physiochemical situations.

Such stochastic development may provide a hypothesis for the formation of the afd cells, which are quite variable with respect to their morphology and number of microvilli. It is interesting, however, that the afd microvilli, once formed are of uniform diameter and directed either anteriorly or posteriorly without deviating much from their direction of travel even though the processes from which they are formed branch variably and can meander and switch directions. This indicates that the direction of growth of the microvilli may be under the influence of some sort of guidance mechanism. In arthropods, it has been determined that the sensory neurons find their way from the epidermis to the interior by fasciculation with existing sensory nerves (Bate, 1978; Edwards and Palka, 1976; Palka, 1979), and the initial sensory contacts are established between the periphery and the center through processes called "pioneer fibers". This describes one of the steps towards the orientation and direction of the outgrowth of neurons, however, it does not address whether the neuronal growth cone is pulled or whether it searches and follows predetermined trails to its destination. Work by Wadsworth and Hedgecock (1992) on the guidance of neuroblast migrations and axonal projections in *C. elegans*, indicates that pioneering axons could be guided by interactions with epidermal cells or muscle cells, and it has been demonstrated that growing neurons preferentially grow along ordered arrays of collagen filaments (Weiss, 1934). One hypothesis put forth by White et al. (1976) is that the defined tracts have a polarity so that growing fibers are restricted to follow them in only one direction, and that cell interactions from existing fibers give the growing processes directional cues. This may explain how the afd dendrites are guided to their final antero-lateral positions in the worm, however, it does not address the issue of how each individual microvillus is directed to grow either anteriorly or posteriorly. There are no

physical links between the microvilli and basement membrane, and the only direct contact that the microvilli make is with the sheath cell plasma membrane into which they project by invagination. One possible explanation for the directionality of the microvilli may then be that the sheath cell provides a physical restraint against the growing microvilli, and the microvilli may simply follow the path of least resistance within the cell which happens to be anteriorly or posteriorly. This is yet to be established, however, such a mechanism would be better described as one of constraint rather than guidance. The constraint could come from the internal cytoskeleton of the sheath cell.

Laser ablation studies by Sulston and White (1980) indicated that with few exceptions the majority of the cells in *C. elegans* appear to be under the influence of autonomous developmental control, however, work by Wood (1991) demonstrates that extensive cell-cell interactions are required in the early stages of embryogenesis for the determination of the many lineal homologues that show left - right differences in their developmental fate. In addition, variations in cell cleavage configurations have been shown, where most cleavages are not completely symmetric (White and Borisy, 1983). This would result in progeny cells that are not perfectly identical in their composition. Morphological variation between the left and right afd's of a worm might be explained by different cell-cell interactions acting on each of the developing afd's, or by variations in any of the cleavages which produce the left and right afd's. Either of these potential causes of left - right variation may be viewed as developmental noise. In addition, variation among either the left or right afd's of numerous isogenic worms may be entirely or partially the result of developmental noise. Since the number of the microvilli of left and right afd's of the same worm are positively correlated it appears that at least one source of developmental noise may affect both sides of the same worm. For example, different feeding patterns, or slight differences in the ambient microenvironments of the worms may be potential causes of variation among the morphology of different worms, however, this is unlikely and small. Waddington (1957) suggested that developing organisms are able to

resist any effects of slight environmental changes. This type of buffering against environmental conditions is called canalization, therefore, it seems rather unlikely that any minute environmental differences, among worms, would affect their development.

One of the main constituents of microvilli cytoskeleton is the protein actin. Tilney and Jaffe (1980) proposed that the formation of *Sea urchin* microvilli begins with the assembly of actin filaments from the plasma membrane, and, with time, adjacent filaments assemble and are "zippered" together. Heintzelman and Mooseker (1990) studied the roles of actin, villin, and fimbrin during the early events of brush border morphogenesis, and found that villin mediates the actin core filament assembly and fimbrin, the primary filament cross-linker of the core, aids in increasing the rigidity and uniformity of length of the microvilli. It appears then that if developmental noise were to affect any or all of the constituents of microvilli formation such as actin, villin or fimbrin, then this may affect the development and/or morphology of the microvilli.

It would be interesting to determine the level of development of the *afd* cells where the first signs of variability become apparent. Some potential levels of development where noise may affect the morphology of the *afd*'s are: (1) the developmental roles of neurotransmitters, where neurotransmitters apparently play prominent roles in shaping the neuronal architecture and connectivity patterns of neurons (Goldberg and Kater, 1989); (2) cleavage configurations of the precursor cells, and; (3) the five main levels of protein (e.g. actin) synthesis: (a) transcriptional control; (b) processing control; (c) transport control; (d) translational control, and; (e) mRNA degradational control. Depending on the protein, and the type of cell in which it is produced, the control of its synthesis may occur at any or all of the above steps, however, it has been illustrated that an important aspect of developmental control is enforced at the transcriptional level (Darnell, J. E., 1982; Derman et al., 1981). These mechanisms of control at the transcriptional level revolve around regulating the amount of primary transcript produced, and are regulated by proteins which either change chromatin structure or, in the case of gene-activator or gene-repressor

proteins, directly facilitate or prevent RNA polymerase attachment to the DNA (Weisbrod, S., 1982). Further quantitative work in these areas may help to provide a better understanding of the degree of precision of the developmental processes.

In many invertebrates the effects of exposure to different conditions of illumination may be seen in the number or degree of order of the photoreceptive microvilli. Such photoreceptive microvilli undergo microvillar degradation and turnover which acts to renew the photosensitive pigment located in the microvillar membrane. The fact that I did not detect a significant difference between the number of *afd* microvilli from light and dark raised nematodes raises the possibility that the *afd* cells may not be involved in a photoreceptive role. However, the background variation present as a result of variation within *afd*'s may mask any slight effects of illumination. Close examination of the transverse sections through the *afd*'s revealed no evidence of microvillar degradation in either the light or dark raised *afd*'s. This indicates that if there are any effects of light or darkness on *afd* development it is not large enough relative to the background variation to be detected with a sample size of 83 *afd*'s.

The fact that there is noticeable variation among the number of *afd* microvilli suggests that a certain degree of variation may be manifest in the behavior of different worms. Studies by Hedgecock and Russell (1975); Mori et al. (unpublished results); and Perkins (1986) indicate that the *afd*'s have a thermoreceptive role, and that there is some degree of variability in the thermotactic behavior of wild-type worms. Hedgecock and Russell showed that worms migrating along a gradient toward their acclimation temperature orient less accurately than worms moving isothermally at their acclimation temperature, and Mori et al. indicate that there is noticeable variation in the isothermal tracking response of different worms. I propose that one possible explanation for this noticed variability in the thermotactic behavior of wild-type worms may reside in the morphological variation of their *afd*'s.

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