Ellsworth C. Dougherty: A Pioneer in the Selection of *Caenorhabditis elegans* as a Model Organism

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ABSTRACT Ellsworth Dougherty (1921–1965) was a man of impressive intellectual dimensions and interests; in a relatively short career he contributed enormously as researcher and scholar to the biological knowledge base for selection of *Caenorhabditis elegans* as a model organism in neurobiology, genetics, and molecular biology. He helped guide the choice of strains that were eventually used, and, in particular, he developed the methodology and understanding for the nutrition and axenic culture of nematodes and other organisms. Dougherty insisted upon a concise terminology for culture techniques and coined descriptive neologisms that were justified by their linguistic roots. Among other contributions, he refined the classification system for the Protista.

KEYWORDS axenic culture; model organism; linguistics; terminology; phylogeny

ELLSWORTH Charles Dougherty (Figure 1) made significant contributions to the foundations of the areas of science that would eventually evolve into molecular genetics, neurobiology, and the genome projects. The year 2015 is the 50th anniversary of his death. Building on observations and insights from as far back as the mid-19th century, the primary core of Dougherty's contributions to science was his recognition of the value of rhabditid nematodes, particularly *Caenorhabditis* spp., as biological models for studies in genetics and many other aspects of biology. He emphasized the importance of culturing the nematodes in the absence of other organisms to facilitate appropriate studies. Those insights drove his intensive efforts to develop chemically defined media, and his collaborative studies on mutants and genetic recombination in nematodes. He reasoned that, if the phenotypic expressions of physiological and developmental mutations in multicellular organisms were to be understood unequivocally, it would be necessary to remove or control all extrinsic sources of variability. Among other things, it would require explicit identification of the characteristics of the diet. That became the driving force of his research for more than 20 years of his scientific career and strongly influenced his students and colleagues. A brilliant man who apparently became depressed as a result of professional and personal disappointments, Dougherty ended his own life on December 21, 1965, at the age of 44.

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Personal History and Attributes

Born July 21, 1921, Dougherty was raised and educated in Berkeley, California. Other than some international collaborations such as a Guggenheim Memorial Fellow between 1947 and 1950, he spent his career in the San Francisco Bay area. Recognized by his peers as a genius who was deeply invested in the ideals of scholarship, Dougherty achieved his AB degree in Zoology at age 18, PhD at age 22, and MD in 1946 at age 25. His interests in nematodes and micrometazoa were already evident when, at the age of 21, he became a member of the American Society of Parasitologists and the Helminthological Society of Washington. At various times he was also a member of the European Society of Nematologists and the American Microscopical Society and was a charter member of the Society of Nematologists (Corliss 1966; Hansen 1966). His PhD dissertation at the University of California, Berkeley, was on the biology of animal-parasitic strongylid nematodes (Dougherty 1944). During his career, he was elected Fellow of the American Association for the Advancement of Science and of the New York Academy of Sciences.

Revered and admired by students, collaborators, and supporters, Dougherty was an "out of the box" thinker, passionate about his science, and a person who did not always

Figure 1 Ellsworth C. Dougherty.

fit comfortably in the collegial academic mold. He was considered impatient and opinionated by some colleagues. In a tribute, parasitologist George Jackson (1966) wrote of the experience of an interaction with Dougherty:

> Pinned down by the man's eyes, you were told the comma, the colon, and the omission of your papers better than you could remember. You were dissected but not discarded. An exchange of letters followed, you reread his works, took exception here and there, but were amazed at the dimensions: morphology and biochemistry, classification and cultivation, evolution of primitive organisms and human disease. If the choices of his topics were diversified and skilled, so were his attacks as natural historian and experimental scientist, medical practitioner, scholar and teacher, neologist and author.

A prolific correspondent, Dougherty's archived files include letters to B.G Chitwood, G. Osche, L.H. Hyman, E.W. Mayr, and other luminaries of the time challenging concepts of evolutionary relationships among micrometazoa and on matters of terminology. He corresponded with Maurice Wilkins and Linus Pauling regarding his ideas on DNA replication (Dougherty 1957–1964, 1961).

When more formal attire was appropriate in a professional setting, Dougherty invariably sported a bow-tie. One of the unique activities of this interesting man on a personal level was that, probably originating as a childhood fascination, he amassed a large collection of first editions of books by L. Frank Baum on the *Wizard of Oz*. Dougherty was an early member of the Wizard of Oz Club in San Francisco. He collected Baum books extensively in the 1950s and 1960s. After his death, his collection was sold successively among private collectors and finally donated to the University of San Francisco, where it is held in the Department of Special Collections at the Gleeson Library (http://ozclub.org).

While working on his PhD at the University of California at Berkeley, he met his China-born wife, Ching-yi Dougherty, who was doing graduate work at Mills College in Oakland, California. The couple married in 1944, being forced to travel to Seattle, Washington, to marry because California still had anti-miscegenation laws at that time. The Doughertys had one son, Brian Shao-lin, born in 1949. Ching-yi Dougherty went on to become Senior Lecturer of Mandarin Chinese at the University of California at Santa Cruz. She retired in 1981 and passed away October 13, 2009 at the age of 96 (Van Den Abbeele 2009; http://news.ucsc.edu/2009/10/3317.html). The Ching-Yi Dougherty Reading Room in the library at U.C. Santa Cruz is named in her honor.

A student of languages—French, German, Russian, Chinese, Japanese, Latin, and Greek—Dougherty commented on and discussed with nomenclatorial commissions details of the names of species and higher taxa (Dougherty 1951c; Corliss and Dougherty 1955; Dougherty and Allen 1958), even challenging Chitwood (1957), through the Commission on Zoological Nomenclature, on the use of the term "nema" (Dougherty 1958a,b). He was frustrated by the ambiguity of terms, such as "pure culture" and "sterile media," used in the culture and maintenance of microorganisms. He introduced more specific terms that became neologisms in general usage, often drawing on earlier literature. For example, the term "axenic" was used by Baker and Ferguson (1942) and "gnotobiotic" was introduced by Reyniers *et al.* (1949); Dougherty introduced xenic, monoxenic, dixenic, etc., as unambiguous descriptors of the numbers of organism types included in gnotobiotic culture, and oligidic, holidic, and meridic as descriptors of the level of knowledge of the precise chemical constitution of the media (Dougherty 1953a, 1959, 1960; Corliss 1966).

In dealing with the need to describe the structure of nuclei for his revision of the Protista, Dougherty drew on some rather rare French publications that divided the group into the procaryotes (those without a definite nucleus and individual mitochondria) and the eucaryotes (those with a nucleus and mitochondria) (Chatton 1925, 1937/1938; Lwoff 1932; Katscher 2004). Interestingly, one of the few copies of Chatton's 1937/1938 book that exist worldwide is in the U.C. Berkeley library (Katscher 2004), but it is uncertain whether Dougherty used that particular volume as a reference source. He proposed the term "prokaryon" for bacterial nuclei not bound by a membrane and "eukaryon" for membrane-bound nuclei of other "primitive" organisms (Dougherty 1957). From that evolution of definitions the terms "prokaryote" and "eukaryote," originating with Chatton (1925), came into general use in modern biology.

Professional Career

The breadth of Dougherty's interests in biology and medicine was vast. Coincident with his various research activities and appointments, between 1952 and 1961 he was a practicing physician in internal medicine with the Kaiser Permanente Medical Group in Oakland, California, with particular concern for the special problems of young people. He pursued and obtained funding that would facilitate the achievement of his research passion. A cursory examination of his resume reveals a lack of permanence and a bewildering array of fellowships, appointments, and affiliations at least during the early part of his research career. From archived letters to granting agencies and colleagues, it is clear that he was frequently concerned about the availability and security of funding to support his research. At times he supported the

work with income derived from his appointment as a physician (Dougherty 1957–1964).

The central locus of his career was always in and around the University of California at Berkeley. During a 1947–1948 National Cancer Institute Postdoctoral Fellowship, he worked in the Berkeley Radiation Laboratory, which was directed by E. O. Lawrence, the 1939 Nobel Laureate in Physics. As a result of that affiliation, he appreciated the potential of radioisotopes and contributed extensively to an early text (Dougherty 1949). Between 1947 and 1949 on a Guggenheim Memorial Fellowship, he was associated with colleagues at the Universities of Paris and Lyon and the Kerckhoff Laboratories of Biology of the California Institute of Technology. Later, he negotiated with directors of the Radiation Laboratory at U.C. Berkeley for access to an unused structure, the old goat barn in Strawberry Canyon, an area later developed into athletic facilities, where he established a laboratory for his work on nematode nutrition.

In 1957 he established the Laboratory of Comparative Biology, which was the founding unit of the Kaiser Foundation Research Institute, in Richmond, California, and was the director of that institute between 1957 and 1962 (Cushing 2012). During that period he collaborated with Mary Bell Allen on pigmentation and the biochemistry of algae (Dougherty and Allen 1956, 1958; Dougherty *et al.* 1957; Allen *et al.* 1959). Interestingly, in 1958 he invited B. G. Chitwood, another genius scholar of the micrometazoa, particularly nematodes, to join the institute as a consultant biologist, Chitwood was at the Institute for 3 years, and it was surely a time during which intellects clashed and sparks flew (Taylor and Esser 1972; Thorne 1972)! Dougherty's separation from the Kaiser Foundation Research Institute was not cordial, and it became necessary for communication with former administrators and colleagues to be conducted through intermediaries (Dougherty 1957–1964).

Sponsored by the National Science Foundation in collaboration with the U.S. Antarctic Research Program, Dougherty participated in three expeditions to McMurdo Sound, Antarctica, in 1959–1960 and 1960–1961 to study biodiversity of the microfauna of the Taylor Dry Valley and other areas. Microfaunae of several freshwater environments were surveyed, and species of nematodes, rotifers, tardigrades, and collembolan were documented (*e.g.*, Dougherty *et al.* 1960; Dougherty and Harris 1963). The study extended into the successful culture and determination of the nutritional requirements of Antarctic organisms (Dougherty 1964a,b). Commemorating his contributions to the success of those expeditions, Mount Dougherty, a 2789-m peak at latitude −82.717, longitude 161.083 in the Queen Elizabeth Range of Antarctica, was named for him (http://en.wikipedia.org/wiki/Mount_Dougherty).

Across the years, Dougherty was sequentially associated with departments of the U.C. Berkeley: Zoology, Physiology, and Medical Physics and, between 1961 and 1965, with Nutritional Sciences. In his Curriculum Vitae document dated May 25, 1964, he indicated that he had held the position of Specialist in Nutrition since 1961 and Lecturer in Nutrition since 1962 in the Department of Nutritional Sciences at U.C. Berkeley. Both were nontenure-track positions (Dougherty 1964c). Recorded memory differs on details of those appointments prior to his death. One version suggests that differences of opinion among factions of the faculty resulted in loss of his position and contributed to his depression and, ultimately, his suicide (Nicholas 1984; Brown 2003). However, in the introduction of his dissertation, W.F.H., Dougherty's last graduate student indicated that at the time of his death Dougherty held the position of Director of the Laboratory of Comparative Nutrition in the Department of Nutritional Sciences (Hieb 1971). Also, W.F.H. recalls some disagreement in the Nutritional Sciences Department about the focus on nutritional requirements of an invertebrate model system.

A Brief History of Research on Nematode Genetics

Ideas on the potential of nematodes for use in genetics research were not original or unique to Dougherty, Nigon, and their colleagues. As is common in the evolution of knowledge, the progression of "standing on the shoulders of giants" applies here because ideas regarding the use of nematodes as model organisms for studying genetics, embryology, and development were evolving much earlier. The investigations of Theodor and Marcella Boveri on the fertilization, cell division, and early embryonic development of horse ascarids (Boveri 1888, 1895) led to the chromosome theory of heredity (Satzinger 2008). The Boveris had built on the pioneering work of Van Beneden and colleagues on the embryology of ascarids (Van Beneden and Neyt 1887). The nematode genus *Rhabditis*, erected by Dujardin (1845), was studied extensively by Schneider (1866), Örley (1886), and others. The embryological stages and fate of the three germ layers in rhabditid nematodes were described at the end of the 19th and beginning of the 20th centuries by Goette (1882), Ziegler (1895), and Neuhaus (1903); postembryonic growth and molting were studied by Maupas (1899). German and French workers continued to make major contributions. Early research on the reproductive strategies, cytogenetics, and embryonic and post-embryonic development of these organisms was conducted by Krüger (1913), Hertwig (1922), Belar (1924), Honda (1925), and Chuang (1962). Nigon and his colleagues (Nigon 1943, 1946, 1949; Nigon and Dougherty 1949a,b; Nigon and Brun 1955) studied the hermaphroditic and sexual forms of reproductive biology of the rhabditids, which were to be important characteristics in the selection of these organisms as biological models.

The biology, life history, ecology, taxonomy, and systematics of the rhabditid nematodes had been progressively documented by Reiter (1928) and, more recently, by Sudhaus (1976) and Andrássy (1983). Schneider (1866) reported the existence of a life stage with a cuticle differing from that in other stages; he considered this form to be a molting stage

but was uncertain of its role. According to Maupas (1899), Pérez (1866) recognized an "encysted" stage in *Rhabditis teres* and indicated that larvae easily encysted at the end of the second stage. Experimentally, Maupas (1899) determined that it was always the same life stage that entered encystment when nutrients were lacking. He noted that encysted nematodes survive for weeks and are often a dispersal stage; he showed that emergence from the encysted stage occurred following nutrient enrichment. Later, Fuchs (1915), in his description of rhabditids associated with bark beetles, coined the term "dauerlarva" for this persistent or enduring "encysted" stage.

Contributions of Dougherty

Dougherty was a visionary and a prolific researcher. Much of his early research with nematodes, before he focused on axenic culture and nutritional requirements of bacteria-feeding forms, was on the taxonomy of animal parasitic species. Early in his career he met George Beadle and Edward Tatum and was intrigued by their work on the biochemical genetics of *Neurospora* and the one gene–one enzyme hypothesis. Recognizing the potential of applying the advances in biochemical genetics to the micrometazoa, particularly the Nematoda, the rationale for his goal of developing chemically defined media was that such techniques would be invaluable in understanding the physiological and developmental phenotypes of mutants and ultimately in understanding their genetic basis. Progress through many years of research in his laboratory was published as abstracts of reports given at scientific meetings of many of the professional societies to which he and his colleagues belonged (*e.g.*, Dougherty and Keith 1951; Dougherty and Hansen 1956a,b), in short papers in *Science* and *Nature* (Dougherty 1950, 1951a), or in more extensive reports as the work developed (*e.g.*, Dougherty *et al.* 1950; Dougherty 1951b, 1953b; Dougherty and Keith 1953; Dougherty and Hansen 1956c, 1957a,b; Hansen and Dougherty 1957). Progress of his work and that of others in the field of nutrition and culture of micrometazoa was collated in detail in extensive proceedings of symposia that he organized (Dougherty *et al.* 1959, 1963; Nicholas *et al.* 1959).

Essentially, Dougherty was in the right place at the right time to recognize and communicate the potential of rhabditid nematodes, including those that would become classified as *Caenorhabditis* spp., in evolving fields of biology. The combination of his foresight, enthusiasm, and passion cast him among the catalysts for selection of the model organism to be used in the many studies and advances that followed. Both Chandler (1924) and Dotterweich (1938) had recognized the potential of rhabditid nematodes as candidates for studies in genetics. The attributes of this group of nematodes as models for the evolution of modern genetics were developed and summarized in a letter to *Nature* by Dougherty and Calhoun (1948b) several years prior to the description of the structure of DNA (Crick and Watson 1953) and before the subsequent evolution of molecular genetics and molecular biology generally (Figure 2). The insights in the letter were reinforced by the work of Nigon and Dougherty (1949a,b) and of Nigon on modes of reproduction in free-living nematodes (Nigon 1949).

The important characteristics of rhabditid nematodes listed by Dougherty and Calhoun (1948b) include the following:

1. Easily cultivated on nutrient agar in the presence of bacteria as a resource;
2. Small size, short life cycle;
3. Relative constancy of a few hundred somatic nuclei allowing determination of the effects of mutations at the cellular level;
4. Few chromosomes and diverse sex patterns that offer a wide range of opportunities for detection, manipulation, and study of mutations, for example: (a) some species dioecious with similar numbers of females and males, others hermaphroditic with few males and yet others parthenogenic; (b) hermaphrodites with XX and males XO chromosome complements;
5. The potential for culture in chemically defined media that would provide the opportunity to study physiological mutants.

Research on *Caenorhabditis elegans* and Other Nematodes

Dougherty's direct involvement and contribution to the subsequent advances in neurobiology and developmental biology, particularly the future work centered on *Caenorhabditis elegans*, was fueled by the fortuitous discovery of a rhabditid nematode on the campus of Stanford University in Palo Alto, California, by Margaret Briggs Gochnauer, in 1944. Gochnauer used the nematode, which she identified as *Rhabditis* sp., in her MS studies of its lifecycle in association with bacteria and in various culture media devoid of other organisms (Briggs 1946). She went on to use this species in studies on the effects of antibiotics (Gochnauer and McCoy 1954), which later became relevant in the development of axenic culture methods.

Dougherty had actually begun to work on nematode nutrition with the sexually reproducing *Rhabditis pellio* (Dougherty and Calhoun 1948a). When he became aware that the nematode studied by Margaret Briggs Gochnauer was a self-fertilizing hermaphrodite, he realized that the impact of variability resulting from genetic recombination would be reduced by such a genetic system, so he switched his studies to that species. Briggs (1946) reported that the nematode could not be sustained in the absence of bacteria or even on dead bacterial cells; living bacteria were a necessary food source. However, survival of individuals was greater on some bacteria-free media than on others. Briggs Gochnauer had attempted to culture the nematode on 12

Gram-negative and 10 Gram-positive bacteria. Six of the Gram-negative but only one of the Gram-positive bacteria supported the nematode in monoxenic culture (Briggs 1946). Dougherty wondered about the differences among these bacteria.

Later, Dougherty and Victor Nigon described the nematode used by Briggs Gochnauer as *Rhabditis briggsae* (Dougherty and Nigon 1949). A related species was earlier described and named *Rhabditis elegans* by Maupas (1900) who collected it from rich humus soil in Algeria (Fatt 1961); the two species were subsequently placed in the subgenus *Caenorhabditis* by Osche (1952). The subgenus was elevated to genus rank by Dougherty (1955a); its name is a blend of Greek and Latin (*Caeno*, recent; *rhabditis*, rod; the specific name *elegans* is derived from elegant). Dougherty's collaboration with Nigon under the auspices of his Guggenheim Fellowship at the University of Paris and later at the University of Lyon was an important period in creating the groundwork for *Caenorhabditis* as a model system. The collaboration of Dougherty and Nigon led to further understanding of the reproduction patterns of Rhabditidae; their work included experiments attempting to hybridize *C. briggsae* and *C. elegans* and the discovery of a dwarf mutant form of *C. briggsae* (Nigon and Dougherty 1950a,b).

Two strains of *C. elegans* were used in the early studies. The Bergerac strain was collected in March 1944 by Victor Nigon from the garden of his home in Bergerac in the southwest of France. The following month he took the nematodes to the University of Paris where he re-initiated his thesis research that had been interrupted by the war (M.-A. Félix, personal communication). Nigon identified the nematode as *R. elegans* in that it conformed to the original description by Maupas (1900) (Nigon 1949; Riddle *et al.* 1997). The Bergerac strain was used in the studies by Nigon and his colleagues and students in France (Nigon and Dougherty 1949a,b; Nigon and Brun 1955; Dion and Brun 1971). The Bristol strain was isolated from mushroom compost near Bristol, England, and was used in a short course on agricultural nematology taught in 1956 by L. N. Staniland of the National Agricultural Advisory Service, London (Nicholas *et al.* 1959; Nicholas 1984). Staniland was an applied nematologist; he published extensively on the nature and control of a variety of nematode problems between 1926 and 1967, including damage associated with rhabditid swarming in mushroom beds. The Bristol strain has been the most important in the explosion of activity on *C. elegans* in molecular genetics and developmental biology.

Warwick Nicholas was Lecturer in the Department of Zoology at the University of Liverpool from 1955 until 1960. In 1957 and 1958 he was on leave as a Traveling Fellow of the British Medical Research Council (MRC) funded by a Rockefeller grant. During the tenure of the fellowship, he worked with Dougherty and Eder Hansen in the Laboratory of Comparative Biology, a part of the Kaiser Foundation Research Institute at U.C. Berkeley. Nicholas took the Bristol strain of *C. elegans* with him to California where Dougherty had previously obtained the Bergerac strain from Nigon. Both strains were established by Nicholas in monoxenic culture on nutrient agar with *Escherichia coli* as a food source in Dougherty's laboratory in 1957 (Nicholas and McEntegart 1957; Nicholas *et al.* 1959; Dougherty 1960; Fatt 1961).

In unsuccessful attempts to make crosses between *C. elegans* and *C. briggsae*, Nigon (1949) and Nigon and Dougherty (1949a,b) discovered that greater proportions of males were produced at elevated temperatures but that the fertility of males produced at higher temperatures was decreased. The Bergerac strain of *C. elegans* becomes infertile at temperatures above 18° while the Bristol strain can be cultured at temperatures up to 25°, although males will not copulate below 20° (Fatt and Dougherty 1963; Nicholas 1984). In perhaps the first study in the Nematoda of the genetics of a physiological character, Dougherty's student, Helene Fatt, conducted mating experiments between males and hermaphrodites of the Bergerac and Bristol strains of *C. elegans*. From their initial studies, they concluded that the heat sensitivity gene segregated as a simple Mendelian recessive and that it was autosomal rather than sex-linked, but their later research indicated that at least three genes are involved (Fatt, 1961, 1966, 1967; Fatt and Dougherty 1963). Those experiments provided a valuable example of the potential for genetics studies with the nematode.

During the early nutrition and neurobiology work in several laboratories, there was confusion and misidentification of cultures of *C. briggsae* and *C. elegans*. The two species are distinguished morphologically by the pattern of rays in the male bursa, but this is a difficult character to use in organisms in which males are rare. In the mid-1970s, graduate student Paul Friedman at U.C. Riverside, after getting inconsistent results while testing antibiotics on *C. briggsae* and *C. elegans*, developed the diagnostic criteria based on isozyme electrophoresis for separating the two species (Friedman *et al.* 1977). The combination of Brenner's (1974) paper on the genetics of *C. elegans* and the misidentification of cultures resulted in many laboratories restricting their studies to *C. elegans* (Riddle *et al.* 1997).

Linkages and Connections

Consider the chain of events associated with the Dougherty–Nicholas–Brenner conduit of information, ideas, and cultures that was facilitated by the Traveling Fellow Rockefeller grant to Nicholas. Sydney Brenner was the mover and shaker at the MRC unit for research on the Molecular Structure of Biological Systems at Cambridge, UK, and was debating the next steps in translating the successes of Watson and Crick into a greater understanding of "life." In 1954, Brenner visited U.C. Berkeley and the Kerckhoff Laboratory at the California Institute of Technology (http://library.cshl.edu/personal-collections/sydney-brenner). It is not known by the authors if he became acquainted with the nematode work during those visits, but it is at least an interesting coincidence that Dougherty had current or recent affiliations

Possible Significance of Free-living Nematodes in Genetic Research

THE free-living nematodes of the sub-order Rhabditina are widespread in the soil[1] and relatively easily cultivable[2-4] on nutrient agar in the presence of bacteria and have short life-cycles (3–7 days from hatching to sexual maturity), with adult sizes up to 3 mm. in length. They offer, in our estimation, certain very interesting possibilities for the study of basic genetic phenomena—morphological, cytogenetic and physiological.

The particular morphological significance of these forms is related to the phenomenon of cellular constancy, or eutely, which the phylum Nematoda (along with the other aschelminth phyla) exhibits for some or all somatic cells. Therefore, the free-living nematodes, containing at most a few hundred somatic nuclei in cells and syncytia, offer material in which mutations affecting structural components may well be interpretable in terms of cellular morphology, rather than only in terms of organ morphology and gross structure.

The cytology of the sex cells of various species of the genus *Rhabditis* Dujardin (1844) has been studied by a few workers[5-11], and has revealed some very interesting sex-patterns. Diploid numbers of 10–24 chromosomes are known for the females and hermaphrodites of different species; the males, where known, are the heterogametic sex with an *XO* sex-chromosome pattern. Some species are dioecious, usually with approximately equal numbers of males and females; others are composed of hermaphrodites of female form and much rarer males, or no males at all; and a few consist only of thelytokous females. Certain dioecious and many hermaphroditic species use their sperm only to initiate development; the sperm confers its centrosome, but not its nucleus, on the ovum[5,7,8]. In some of these species pairing and reduction of chromosomes nevertheless occurs in meiosis; the diploid number is reconstituted with the failure of a second meiotic division to occur. Sex-determination is apparently effected by an unusual regulation of *X*-chromosome behaviour in meiosis. With simple chromosome numbers these forms should be good cytogenetic material; and with their unusual sex-patterns a considerable versatility in the detection and manipulation of mutations should be possible.

Another important consideration is the possibility of studying physiological mutants. As a result of the work of Kidder and Dewey[12] it is now possible to grow at least one organism of animal nutrition (the ciliate *Tetrahymena geleii*) on an almost completely chemically defined medium (consisting of inorganic salts, glucose, vitamins, amino-acids, purines and pyrimidines, and one unknown growth factor). It seems certain that the near future will see a completely known synthetic medium for this form. Through the use of a chemically defined medium the possibility arises that the new concepts of physiological genetics developed by Beadle[13] may be tested on a differentiating organism. Kidder and Dewey's work suggests a valuable lead for the development of a chemically defined medium for species of *Rhabditis*.

We feel that, with eutely, good cytological features, and convenient sex patterns, together with promising cultural and nutritional aspects, the soil-dwelling nematodes offer attractive possibilities for a correlation and precise interpretation of the morphological and physiological genetics of a simple, differentiating organism. Such a simultaneous approach is being attempted in this laboratory. We are presenting the foregoing discussion in the hope of stimulating others to work on the same problem.

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Figure 2 The letter to the journal *Nature* by Dougherty and Calhoun (1948b) detailing the potential for use of rhabditid nematodes in genetics research. Note that the letter predates the erection of the subgenus *Caenorhabditis* by Osche (1952) and its elevation to genus level by Dougherty (1955a). Reprinted by permission from Macmillan Publishers Ltd: Nature No. 4079 Page 29, 1948.

with both institutions at that time. Based on the work of Dougherty, Nigon, and their colleagues, Brenner initially proposed to use *C. briggsae* as his model organism and indicated that in his proposal to the MRC in 1963 (Brenner 1988). He established cultures of nematodes from his own garden and from soil samples collected by colleagues. One of those cultures, established with nematodes collected from a compost heap in June 1964, was designated N1 (where N indicates "Nematode"). The culture of N1 was not a *Caenorhabditis* but was identified as *Mesodiplogaster lheritieri*

(Brenner 1966; M.-A. Félix, personal communication). After discussions with Dougherty during a visit to U.C. Berkeley, Brenner switched his interest from *C. briggsae* to *C. elegans* (Brenner 1974; Riddle *et al.* 1997; Brown 2003). In a letter to Dougherty dated October 11, 1963, Brenner requested a culture of *C. elegans*. The culture of the Bristol strain, originally collected by Staniland, which Dougherty sent to Brenner was designated N2 (Ankeny 2001; Friedberg 2010) (Figure 3).

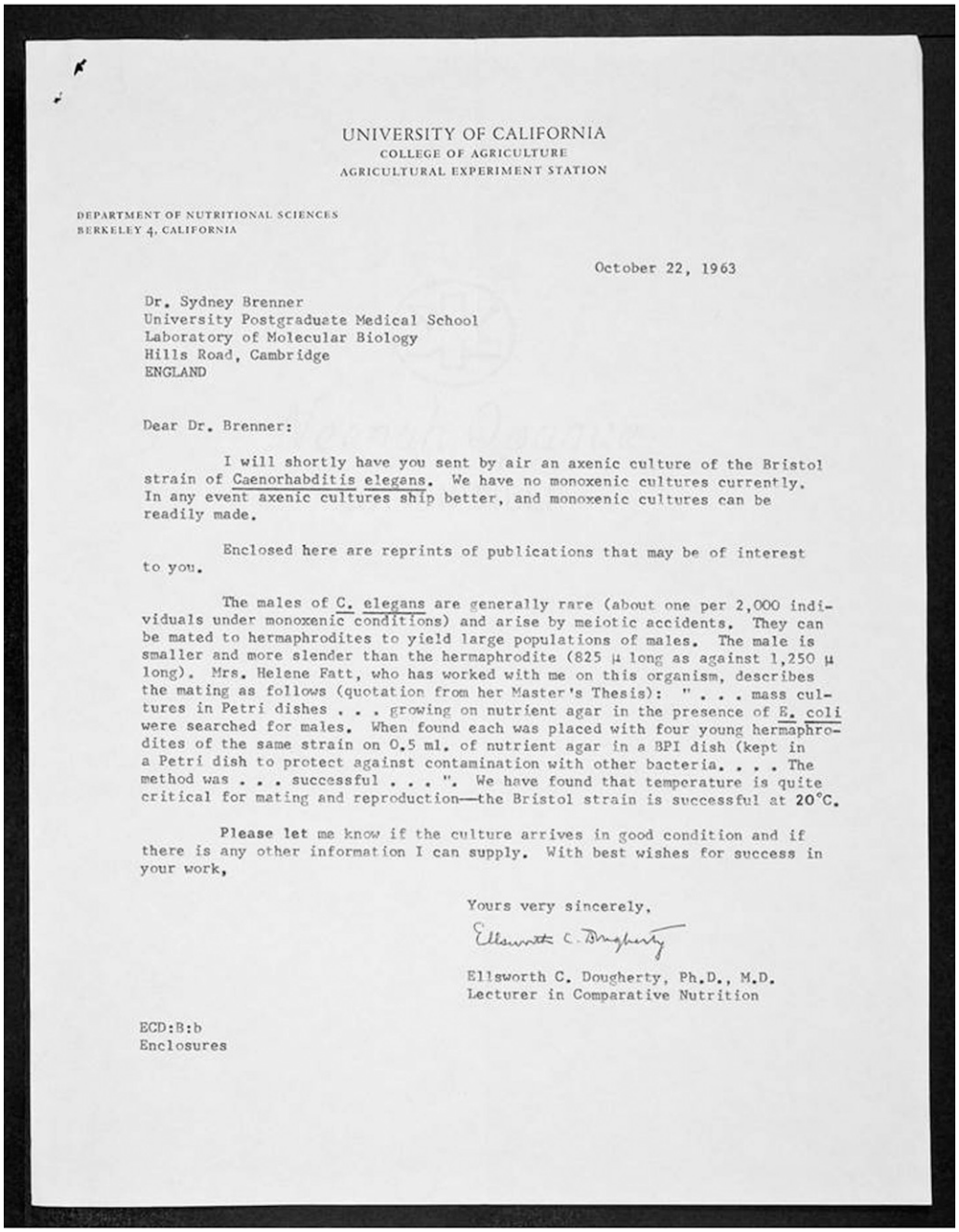
The reasons for the switch from *C. briggsae* to *C. elegans* are now somewhat obscure, but perhaps the most appealing is Brenner's reported quip that he considered *C. elegans* to be more photogenic (Davies 2002). More likely is that Dougherty pointed out differences in the growth rates of the two species (Félix 2008) and possibly the advantageous behavioral attributes of the Bristol strain of *C. elegans*, which were later associated with the *npr-1* gene. Individuals with the *npr-1* gene do not clump or burrow into an agar medium, thus greatly facilitating microscopic observation of behavioral and developmental phenomena. Since the gene does not occur in *C. elegans* populations isolated in nature, it is possible the *npr-1* is a spontaneous mutation that occurred during prolonged culture in Dougherty's laboratory (McGrath *et al.* 2009). Virtually all the studies on *C. elegans* genetics and development have been done with the N2 Bristol strain that Sydney Brenner established from the culture he obtained from Ellsworth Dougherty. The importance of the early studies by Dougherty and colleagues is recognized in Brenner's landmark paper (Brenner 1974).

In association with the early realizations of the scientific value of nematodes of the genus *Caenorhabditis*, Dougherty set out to develop a chemically defined culture medium (Dougherty and Calhoun 1948b; Nicholas 1984). In studies decades earlier, Conte (1900), Reiter (1928) and Clapham (1930) had shown that the morphometrics of these nematodes were affected by the nutritional nature of the culture medium. Through myriad experiments, Dougherty and colleagues developed a chemically defined medium composed of 19 essential and nonessential amino acids, 13 vitamins, six growth factors, five nucleotides, nine salts, and glucose as an energy source (Buecher *et al.* 1966; Hieb 1971). However, the medium would not support reproduction of *C. briggsae* without small additional amounts of substances of undefined chemical characteristics as supplied, for example, by chick embryo, liver proteins, and human plasma (Dougherty *et al.* 1959; Nicholas *et al.* 1959; Sayre *et al.* 1963; Hieb 1971).

Determination of the chemical identity of the final necessary components proved difficult (Nicholas 1984). After Dougherty's death, the work on nutritional requirements of *C. briggsae*, and eventually on other nematode species, was continued by various colleagues, their students, and associates (Hansen and Berntzen 1969; Buecher *et al.* 1970; Hieb 1977). Chemical definition of the amendments was pursued W.F.H., who had begun working with Dougherty as a laboratory helper and who became his close friend and graduate student. The undefined components of the medium were determined to be an iron porphyrin and a sterol. The iron porphyrin could be supplied by either a heme protein or unbound hemin and the sterol by cholesterol or β-sitosterol (Hieb and Rothstein 1968; Hieb *et al.* 1970; Hieb 1971; Nicholas 1984). One of Dougherty's last publications was as co-author of an abstract with W.F.H. (Hieb and Dougherty 1966), who attempted to counsel him through his descent into depression. Dougherty saw W.F.H. as a protégé who would carry on the nutrition work.

Although nematodes can be successfully cultured on various formulations of an axenic holidic diet, populations grow rather slowly and their maintenance in monoxenic cultures with *E. coli* cultures is usually preferred and has been used in most *C. elegans* research (Stiernagle 1999). The culture of rhabditid nematodes has continued to be of interest and importance with the evolution of the commercial application of the genera *Heterorhabditis* and *Steinernema* as biological control agents of insect pests. Interestingly, when produced in axenic culture, these nematodes are generally less infective of their insect prey than when produced in monoxenic culture (Ehlers *et al.* 1997), and sophisticated monoxenic bioreactor systems have been developed (Chavarría-Hernández *et al.* 2011). However, the conventional wisdom is that nematodes are the only animals for which there is a totally synthetic defined medium in which they are able to reproduce continuously.

Dougherty's experiences while working on the pigmentation and biochemistry of algae led to a proposal for a phylogeny of the Protista that included the Monera (bacteria and blue-green algae), Mesoprotista (red algae), and Metaprotista (eukaryotic algae, fungi, and Protozoa) (Dougherty and Allen (1960). He also explored his interests in the evolution of sexual reproduction and suggested that the Protista should be an important group for determining its origins (Dougherty 1955b, 1956). Dougherty corresponded extensively with 1955 Nobel Laureate Joshua Lederberg, a contemporary of Beadle and Tatum. The preserved correspondence (http://profiles.nlm.nih.gov/ps/retrieve/Series/735) includes letters in which Dougherty inquired about mechanisms of genetic recombination among bacteria, particularly *E. coli*, and explained his own observations and ideas. Lederberg invited Dougherty to spend a month in his laboratory at the University of Wisconsin to further discuss the ideas and approaches to testing them. Dougherty responded to the invitation by explaining that he had used up all his vacation time, had no funding, and was supporting his research on salary from his "day job" as a Kaiser Permanente physician! He published his hypotheses and observations on the origin of sexuality during the same period (Dougherty 1955b, 1956). During the latter part of his career, Dougherty expanded his work on nutrition and culture to other organisms, including Rotifera, Tardigrada, Gastrotricha, Copepoda, Turbellaria, and



UNIVERSITY OF CALIFORNIA
COLLEGE OF AGRICULTURE
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DEPARTMENT OF NUTRITIONAL SCIENCES
BERKELEY 4, CALIFORNIA

October 22, 1963

Dr. Sydney Brenner
University Postgraduate Medical School
Laboratory of Molecular Biology
Hills Road, Cambridge
ENGLAND

Dear Dr. Brenner:

I will shortly have you sent by air an axenic culture of the Bristol strain of Caenorhabditis elegans. We have no monoxenic cultures currently. In any event axenic cultures ship better, and monoxenic cultures can be readily made.

Enclosed here are reprints of publications that may be of interest to you.

The males of C. elegans are generally rare (about one per 2,000 individuals under monoxenic conditions) and arise by meiotic accidents. They can be mated to hermaphrodites to yield large populations of males. The male is smaller and more slender than the hermaphrodite (825 μ long as against 1,250 μ long). Mrs. Helene Fatt, who has worked with me on this organism, describes the mating as follows (quotation from her Master's Thesis): " . . . mass cultures in Petri dishes . . . growing on nutrient agar in the presence of E. coli were searched for males. When found each was placed with four young hermaphrodites of the same strain on 0.5 ml. of nutrient agar in a BPI dish (kept in a Petri dish to protect against contamination with other bacteria. . . . The method was . . . successful . . . ". We have found that temperature is quite critical for mating and reproduction—the Bristol strain is successful at 20°C.

Please let me know if the culture arrives in good condition and if there is any other information I can supply. With best wishes for success in your work,

Yours very sincerely,

Ellsworth C. Dougherty

Ellsworth C. Dougherty, Ph.D., M.D.
Lecturer in Comparative Nutrition

ECD:B:b
Enclosures

Figure 3 A letter from Dougherty to Brenner accompanying the requested axenic culture of the Bristol strain of *C. elegans* (Wellcome Library; original held by Cold Spring Harbor Laboratory Archives and Genentech Center for the History of Molecular Biology and Biotechnology). Permission granted by Sydney Brenner Collection. Cold Spring Harbor Laboratory Archives.

enchytraeid annelids (Dougherty 1957–1964; Dougherty *et al.* 1963).

Impacts on Biological Research

The characteristics of rhabditid nematodes, as pointed out by Dougherty and Calhoun (1948b) (Figure 2), and by others both earlier and later, were substantiated and proved invaluable in the work of Brenner, his colleagues, and successors. The MRC Laboratory of Molecular Biology in Cambridge, UK, and the establishment of the *Caenorhabditis* Genetics Center at the University of Missouri in Columbia provided loci for the interaction and productivity of myriad worm specialists, many of whom probably knew nothing of Dougherty. Their activities contributed to the detailed documentation of many aspects of the biology and behavior of *C. elegans*. The nematode was the first multicellular organism to have its genome fully sequenced, and the success of that genome project greatly influenced interest, attention, funding, and understanding of the potential of the human genome project (Lewin 1990; *C. elegans* Sequencing Consortium 1998; Ankeny 2007). Incidentally, in 2003, the genome sequence of *C. briggsae*, the nematode found by Margaret Briggs Gochnauer, described by Dougherty and Nigon, and used by Dougherty for nutritional studies, was also completed (Stein *et al.* 2003). Through studies of the life history

and habits of *Caenorhabditis* in nature has come the understanding that these nematodes are rarely found in soil but rather are inhabitants of decaying fruits and other vegetation and are often dispersed by snails (Félix and Braendle 2010; Kiontke *et al.* 2011). Recognition of the association with decaying vegetation has led to the recent description of 16 new species in the genus on the basis of combinations of mating, morphological and molecular evidence, and the naming of a species discovered in rotting fruit in Kerala, India, as *C. doughertyi* (Félix *et al.* 2014).

The successes associated with the selection of *C. elegans* as a model system have resulted from a combination of design and luck (Hodgkin 1989), but many of the attributes that have been important are among those first detailed by Dougherty and Calhoun (1948b). Among the attributes are that the nematode is small, anatomically simple, easy to culture, and easy to manipulate genetically because of its reproductive strategies and that it has the full range of differentiated cell types of more complex organisms. The genome is the smallest of those known for the Metazoa, which facilitated construction of its complete physical map (Sulston and Brenner 1974; Coulson *et al.* 1988). The nematode is viable after storage in liquid nitrogen, which permits preservation of the pedigree parentage of any mutant line; it is amenable to electron microscopy and has transparent optical qualities that permit high-resolution Nomarski microscopy of living individuals. Finally, there was important background knowledge available from the work of Dougherty, Nigon, and others that facilitated selection of *C. elegans* above other candidate organisms and accelerated Brenner's (1974) development and description of morphological and behavioral mutant lines (Hodgkin 1989).

Consider the linkages and connections in and after Dougherty's rather short career. His thinking and research activities were variously influenced by his meeting and interaction with four Nobel Laureates—E.O. Lawrence, G.W. Beadle, E.L. Tatum, and J. Lederberg. Subsequently, his work and insights directly influenced the science of three other Nobel Laureates and, less directly, two others who worked on or with *C. elegans*: the 2002 prize in Physiology and Medicine of J.E. Sulston, H.R. Horvitz, and S. Brenner; the 2006 prize in Physiology and Medicine of A.Z. Fire and C.C. Mello and the 2008 prize in Chemistry of M.L. Chalfie (http://www.nobelprize.org/nobel_prizes/lists/all/index.html). Major advances in our understanding of living systems have resulted from the field of science in which Dougherty so insightfully participated; important applications in medicine are continuing to emerge.

Final Thoughts

There are few remaining who have living memory of Ellsworth Dougherty. Although intrigued by the nature, activities, and contributions of the man, H.F. never met him. Their careers overlapped by only a few years. W.F.H. provided a wonderful direct link to the history but, even for him, some details have faded. Every stone turned over in researching this article revealed fascinating information, both direct and tangential to the theme; many led to other stones to turn over, always with recognition of the constraint of maintaining focus. We hope that, whatever the gaps may be, our account will revive recognition of the work of this now largely forgotten pioneer.

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