Perspectives

Kenneth Raper, Elisha Mitchell and *Dictyostelium*

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1. Introduction

Dictyostelium discoideum has become an important organism in the study of modern cellular and developmental biology (Kessin 2001). The most recent International *Dictyostelium* Conference, held outside Grenoble, France last summer, was attended by more than 150 scientists from thirteen countries. *D. discoideum* is a member of a group of organisms known as the cellular slime moulds. Cellular slime moulds were first described in the second half of the nineteenth century but their potential as model organisms only began to be appreciated with the isolation of the *D. discoideum* species by Kenneth Raper in 1933. Raper was, at the time, working for the United States Department of Agriculture as a mycologist. It is not surprising therefore, that the first description of *D. discoideum* was published in the *Journal of Agricultural Research* (Raper 1935). In 1937 he published a second paper (Raper 1937) in the same journal, describing the growth and development of *Dictyostelium* with different bacterial hosts, and in 1939, a third paper detailing conditions for growth and development of *Dictyostelium* (Raper 1939a), again in the same journal. In 1940 he published the results of several years of experiments in which he brought the techniques of experimental embryology to bear on *Dictyostelium* (Raper 1940). In a series of elegant studies involving the cutting and grafting of intermediate stages in *Dictyostelium* development, Raper established all the principles that would make *Dictyostelium* a powerful model system for cellular and developmental biology.

So what exactly did Raper do? Figure 1 shows the life cycle of *D. discoideum* that Raper had already worked out. Spores germinate to amoebae which eat bacteria and multiply. When the amoebae run out of food, they aggregate together by chemotaxis to form a mound of cells in about eight hours. This mound then goes through several morphogenetic changes and forms a slug, which can migrate on the surface of a Petri dish for several hours, depending on the environmental conditions. It then goes through more morphogenetic changes, lifts itself off of the substratum and forms the fruiting body. The fruiting body, a small plant-like structure about a millimetre tall, basically consists of only two kinds of cells, stalk cells in the stalk and spore cells contained in a droplet of liquid above the stalk. Although modern work has shown that there are several cellular subtypes, Raper's view was essentially correct. Furthermore, under a given set of nutritional and environmental conditions, the ratio of spore cells to stalk cells in the fruiting body is fixed, with spores representing about 80% of the cells and stalk cells 20%. Since both spore cells and stalk cells derive from amoebae, Raper was interested in understanding how the amoebae made the decision about which of the two cell types to become. He thought it could be a model for how cell fate decisions are made during the embryogenesis of higher organisms. In the Elisha Mitchell paper (Raper 1940), he began to approach the problem experimentally.

One of the many interesting questions he addressed in the paper was whether the position of cells in the intermediate slug structure could affect cell fate. Figure 2, taken from the 1940 paper (Raper 1940), shows his experiment. Raper had discovered that if he grew amoebae on the red bacterium *Serratia marcescens*, he could produce red amoebae which would aggregate and produce red slugs. Amoebae grown on colourless bacteria would produce colourless slugs. Now he did a grafting experiment. He cut a red slug and a colourless slug in two, and grafted the red front onto a colourless rear and visa versa. Each grafted slug went on to produce a fruiting body, and he saw that the grafted slug with a red front produced a fruiting body with a red stalk, while the grafted slug with the red rear produced a fruiting body with red spores. Thus, Raper was able to show that the position of amoebae in the slug did, in fact, correlate with their fate. Amoebae in the front of the slug became stalk cells while amoebae in the rear of the slug became spore cells. The pattern in the fruiting body was already established in the slug. Therefore, to understand how amoebae decide whether to become spore cells or stalk cells, all you have to do is understand how the pattern in the slug is established. Hundreds of scientists have spent the

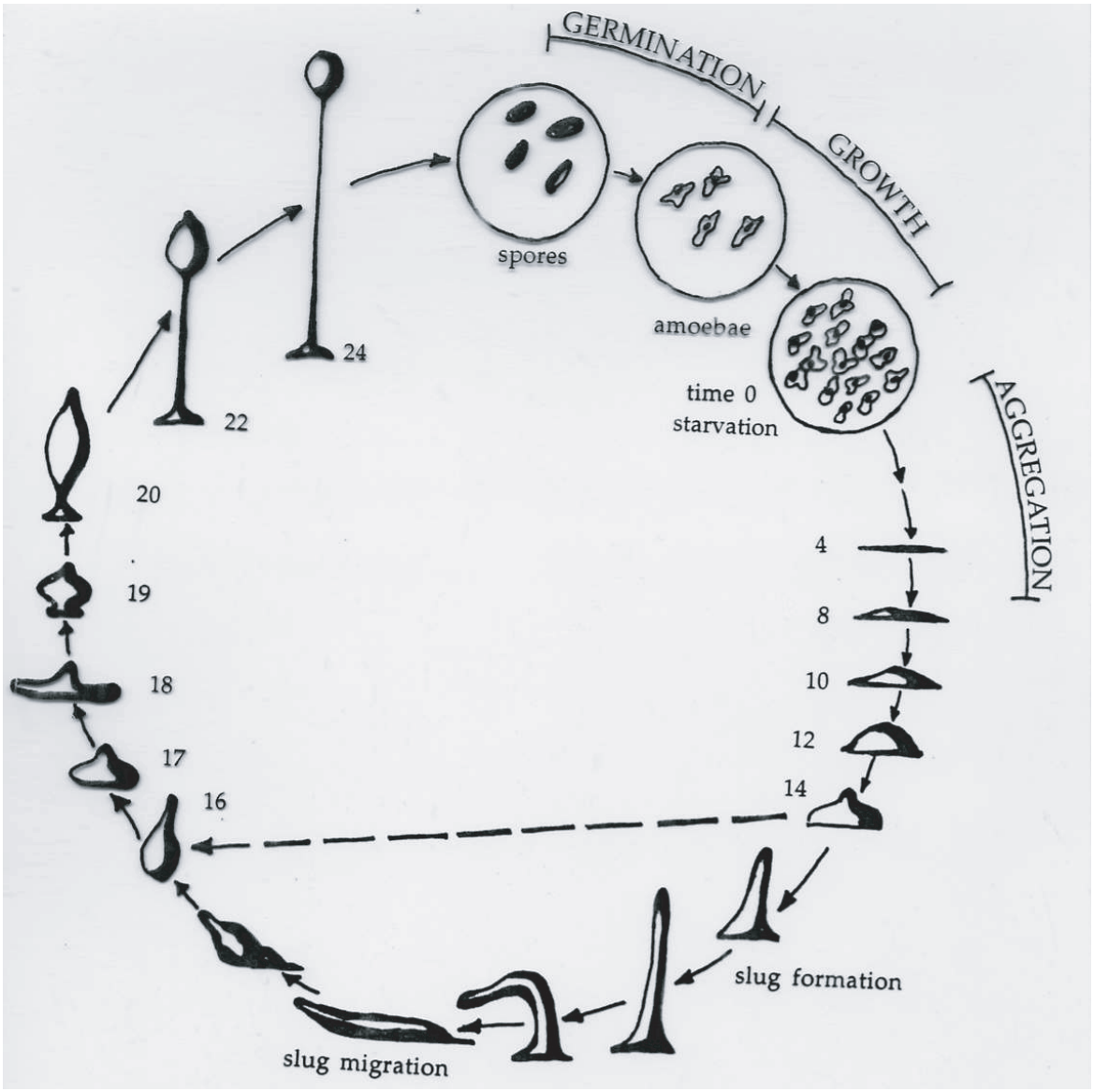

Figure 1. Life cycle of *D. discoideum.* The time is indicted in hours after starvation.

last sixty five years trying to answer that question, and we still have no definitive answer. The one thing that is clear, however, is that all of the work on the organism over these sixty five years follows directly from this monumental 1940 paper. Not only is it the most important paper that has ever been published in the field, but it stands, even today, as one of the great papers in the biological sciences in the twentieth century. It should be read by everyone who wishes to know what beautiful science is all about.

At the time that Raper wrote it, he was one of the few people in the world working on cellular slime moulds and the only one working on *D. discoideum*. It is clear that he appreciated the importance of the work. In December 1939 he was invited to present a paper at the AAAS Meeting in Columbus, Ohio, and in a letter to a colleague (Raper 1939b) he wrote "I shall do my utmost to convince the audience in Columbus that these organisms are unique in the possibilities for study that they afford". Later in the same letter he writes: "I am enclosing a reprint of the paper dealing with the influence of cultural conditions upon the growth and development of *D. discoideum"* (the 1939 paper). "Now that this is out, the way is clear to publish what to me is the more interesting work on the formation of fruiting structures, including grafting experiments, etc." (what would be the 1940 paper). Obviously, Raper did a good job in Columbus, and, with the publication of the great

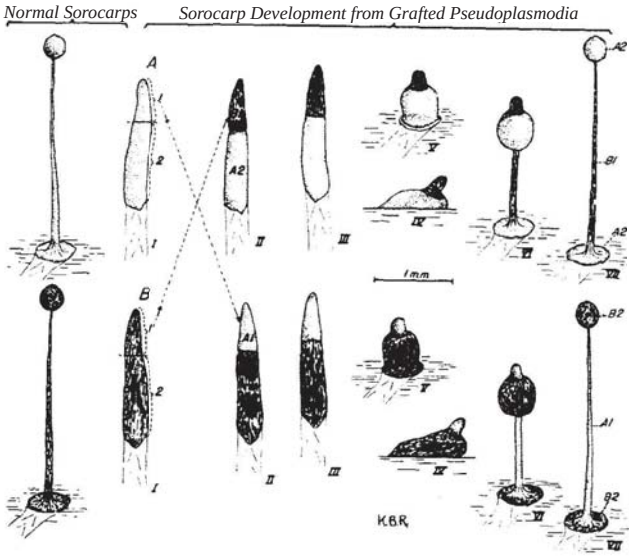

Figure 2. Taken from the Elisha Mitchell paper, shows his experiment. (Raper Kenneth B: THE DICTYOSTELIDS © 1984 Princeton University Press. Reprinted by permission of Princeton University Press.)

Figure 3. Elisha Mitchell (1793–1857). *Courtesy*: The North Carolina Collection, University of North Carolina Library.

1940 paper, the field of *D. discoideum* research began. So where did Raper chose to publish his *magnum opus*? The *Journal of the Elisha Mitchell Scientific Society*, which is one of the most obscure journals in the literature. When I am lecturing to graduate students about *Dictyostelium* and put up that Raper reference, it invariably draws chuckles from them. I have always been curious about why he published there. I met Raper, but never asked him. One of my colleagues told me that he had asked Raper why he had published there, and was told that he did so because he wanted to support the journal. My colleague didn't know what that meant, but I believe that I do now. Fortunately, the Kenneth Raper Archive is maintained at the LuEsther T Mertz Library of the New York Botanical Garden, and a search of the Archive was the starting point of my effort to understand his reasons. In the end, four questions need to be addressed. Who was Elisha Mitchell, what was the Elisha Mitchell Scientific Society, who was Kenneth Raper, and what are the connections between all of the above?

2. Elisha Mitchell

Elisha Mitchell (figure 3) was born in Washington, Connecticut in 1793 and was educated at Yale University (Watson 1984). In 1818 he was hired as Professor of Mathematics and Natural Philosophy (Physics) at the University of North Carolina, Chapel Hill (UNC). UNC had only recently been founded in 1795, and, at the time of Mitchell's arrival had only 100 students. He was considered one of the most educated men in North Carolina at the time, and in addition to Chemistry, Geology and Mineralogy, occasionally taught Greek, Latin, Rhetoric and History. His real claim to fame, however, was as an explorer. In 1825, when a colleague, who had begun a geological survey of North Carolina left UNC, Mitchell was appointed to replace him. In that role he began to explore the mountains of Western North Carolina. It had been suggested that some of the peaks might even be taller than Mount Washington, New Hampshire, then believed to be the highest point in the East. Local politicians were eager to promote North Carolina. It was thought that if it could be established that there was a mountain in the state that was higher than Mount Washington, it would provide bragging rights to the state and a great boost to its self-image, not to say its economy. In 1835 Elisha Mitchell set out to measure the highest peak in the Black Mountains. On July 27, he stood on Yeats Knob (Silver 2003) and surveyed the set of peaks that lay before him. He picked out the highest and the next day he climbed it and measured its height as 6,476 feet. Mount Washington was listed as 6,234 feet. Mitchell's discovery was quickly recorded in the local newspapers and he became an instant celebrity. In subsequent remeasurements in 1838 and 1844, Mitchell refined his estimate to 6,672 feet, only 12 feet below modern measurements.

In 1855, Thomas Lanier Clingman, a local Congressman, who was also a geologist, challenged Mitchell's claim and suggested that Mitchell had measured the wrong mountain, and that it was he, Clingman who had actually measured the

highest peak in 1855. The controversy raged for two years, and in 1857 Mitchell determined to retrace his earlier trips to prove that he was correct. By then Mitchell was 64 years old, which by today's standards does not seem very old, but which was probably seen as quite elderly in 1857. On July 27, while climbing alone, Mitchell disappeared. Search parties found his body 11 days later in a pool of water at the base of a forty foot waterfall. He apparently had been using a stream to guide him and had lost his footing at the top of the waterfall and had fallen to his death. His pocket watch, which is housed as part of the North Carolina Collection at the UNC Library, reads 8:19:56, presumably the time of his death. He was buried in Asheville, North Carolina. In death Mitchell became even more of a folk hero than when he was alive. He was the scientist who had given his life in the pursuit of truth, and for the people of North Carolina. His colleagues and friends took up his cause, and after much lobbying by friends and family, in early 1858 Elisha Mitchell's body was moved to the top of the highest peak in the Black Mountains, the highest peak east of the Mississippi River in the United States, which has ever since been known as Mount Mitchell. The inscription on the monument on the top reads: "Here lies in hope of a blessed resurrection the body of the Rev. Elisha Mitchell, D.D. who, after being for 38 years a professor in the University of North Carolina, lost his life in the scientific exploration of this mountain in the 64th year of his age, June 27th 1857".

3. The Elisha Mitchell Scientific Society

The Elisha Mitchell Scientific Society was formed in 1883 under the leadership of the President of North Carolina to honour the memory of Elisha Mitchell (Mitchell Society Inventory 2003). It was composed of faculty, staff, and alumni of UNC's science and mathematics departments and its purpose was to foster scientific research and the dissemination of scientific knowledge. The Society held meetings where papers were presented and which were subsequently published in the Society's journal, *The Journal of the Elisha Mitchell Scientific Society*. By 1900 research papers were being solicited from leading scientists. By the second half of the twentieth century the Society had become somewhat of an anachronism, with large numbers of discipline-specific societies and journals having come into existence since its inception. In April 1983, at the Society's Centennial Celebration , the Elisha Mitchell Scientific Society was officially dissolved and the publication of its journal passed to the North Carolina Academy of Sciences.

4. Kenneth Raper

Kenneth Raper (figure 4) was born in Welcome, North Carolina in 1908 (Raper 1986; Burris and Newcomb 1991)

Figure 4. Kenneth Raper. Reprinted by permission of the LuEsther T Mertz Library of the New York Botanical Garden, Bronx, New York.

He entered the University of North Carolina, Chapel Hill in 1925, and graduated as a botany major, with a history minor. Before graduating, he took a civil service exam for a position as Junior Mycologist in the US Department of Agriculture (USDA)'s newly formed Division of Soil Microbiology in Washington DC. The head of the lab, the eminent mycologist Charles Thom, hired Raper after interviewing him on a trip to Chapel Hill. The two became, and remained, close collaborators until Thom's death in 1956. Thom encouraged Raper to take additional courses in biology and bacteriology which Raper did in the Evening Division of George Washington University where Raper got a Master's Degree in 1931. Thom's lab was mainly interested in *Penicillium* and *Aspergillis* species and Raper became an expert on these moulds. By 1933 Thom was encouraging Raper to continue with his graduate studies, and suggested Harvard, where he had some contacts. Raper took a leave of absence from the USDA, and went off to Cambridge, Mass. where he did his Ph. D. research under the direction of Professor William Weston. In 1936, with Ph. D. in hand, Raper returned to Thom's lab as an Assistant Mycologist, with a $200-a-year raise. There he continued work on *Penicillium* and *Aspergillis*, work which culminated some years later in the

publication of the *Manual of the Aspergilli* (Thom and Raper 1945) and the *Manual of the Penicillia* (Thom and Raper 1949). In 1940 Raper moved to the newly opened Northern Regional Research Laboratory in Peoria, Illinois, whose purpose was to find new products that resulted from the fermentation of farm commodities. Within a year, however, Raper's full energy had turned to efforts to produce commercial quantities of penicillin. The success of this work (see below) saved the lives of thousands of troops in World War II and led to Raper's sharing the Lasker Prize in 1946. In 1952 Kenneth Raper moved to the University of Wisconsin, Madison, where he remained for the rest of his academic career, with a joint appointment in Bacteriology and Botany. From 1966 until his retirement in 1987, he held the William Trelease Professorship in Bacteriology and Botany. During his life Kenneth Raper received many honors in addition to the Lasker Award. He was the first recipient of the Charles Thom Award, given by the Society of Industrial Microbiology in 1967. He received the Certificate of Merit from the Botanical Society of America in 1960 and the Distinguished Mycologist Award from the Mycological Society of America in 1981. He was an Honorary Member of the American Society for Microbiology and the British Mycological Society. He was elected to the National Academy of Sciences in 1949, and received an Honorary Doctor of Sciences Award from the University of North Carolina in 1961.

5. So why did Kenneth Raper publish his seminal paper in the *Journal of the Elisha Mitchell Scientific Society*?

What stands out from the above discussion is the University of North Carolina connection. Elisha Mitchell was a Professor there for many years, and the Elisha Mitchell Scientific Society was established and based there. Kenneth Raper was an undergraduate at UNC. Although the Elisha Mitchell Scientific Society no longer exists, the Archive of the Society is maintained at the University of North Carolina Library. I was able to persuade the Archivist to examine the Society's membership list from 1925-1929 and she reported back to me that Kenneth Raper was an Associate Member of the Society in 1927, 1928 and 1929 (Mitchell Society Archive 2003). While still an undergraduate he published his first paper, on water moulds, in the *Journal of the Elisha Mitchell Scientific Society* (Raper 1928).

But there is more. In the summer of 1933, before Kenneth Raper began his Ph. D. studies at Harvard, he went home to North Carolina for a holiday. As part of that vacation he went hiking in the mountains in Western North Carolina. Ever the mycologist, Raper collected eighteen samples from that trip, and wrote a detailed account (Raper 1933) describing where each sample was taken. On June 22, 1933 Ken started his hike by climbing Yeates Knob. This is the exact place, where, 98 years earlier , Elisha Mitchell had looked out over the Black Mountains, picked out the highest peak, which he climbed and measured the next day, and which is today Mount Mitchell. Raper then hiked along a trail which followed along the crest of a very steep ridge. About a mile and a half from Yeates Knob, according to Raper, the ridge drops off abruptly and soon enters Little Butts Gap (Silver 2005). It was here that Raper collected the fourth sample from the hike and it was in that one that he found *D. discoideum* (Raper 1984). Raper named the strain NC-4, and it is the basic wild-type strain that has been used by hundreds of laboratories around the world ever since. Raper took *D. discoideum* with him to Harvard and persuaded his professor there to allow him to study it for his Ph. D. research. Little Butts Gap is four miles from Mount Mitchell and three and a half miles from Mitchell Falls, the waterfall where Elisha Mitchell lost his life. In choosing to publish his great work in the Elisha Mitchell Journal, therefore, I believe that Kenneth Raper had two reasons. The first, already mentioned, was his connection to the University of North Carolina and the Elisha Mitchell Society. The second is my guess that Kenneth Raper was fully aware of the proximity of the place where *D. discoideum* was discovered to the places that were so important in the life and death of Elisha Mitchell.

6. Kenneth Raper and penicillin

There was more to Kenneth Raper than the discovery of *D. discoideum.* From 1941 until 1945, he played an important role in the development of penicillin as the first important antibiotic. Raper remembered that time in 1978 (Raper 1978) and the description below is excerpted from his article.

In 1929 Alexander Fleming reported that a strain of *Penicillium* could produce an antibacterial substance. He named it penicillin and showed that the substance seemed non-toxic to laboratory animals. He speculated that the substance might have utility in treating some bacterial infections, but his work really stopped there. With the outbreak of World War II it was taken up again by a group at Oxford University led by Howard Florey. They extended Fleming's work to the point where they actually treated patients with penicillin and reported promising results. They were limited, however, by the difficulties in culturing the mould and the small yields of penicillin that were produced. They could imagine the production of commercial quantities of penicillin, but they thought that it could not be done in England which was being bombed. In July 1941 Howard Florey and his colleague Norman Heatley came to the US for help. They were directed to talk to Charles Thom in Washington who was the world's expert on *Penicllium.* (It is interesting to note that in the printed version of Raper's article Thom's name is continuously misspelled as Charles Thorn.) Thom recommended that they go to the Northern

Regional Research Lab in Peoria, Illinois. That is where Kenneth Raper, along with a substantial part of Thom's mould collection, had moved the previous year. A three-pronged research effort on penicillin began immediately. One group sought to develop better culture media for the mould to generate higher levels of penicillin. A second group sought to find conditions where the mould strain could grow in submerged culture conditions. At that point the mould would only grow as surface cultures, which required enormous numbers of flasks. Florey had been using bedpans in England. The third research group, headed by Kenneth Raper, was to find or develop more productive strains of *Penicillium*. In the end, all three groups were successful.

Raper's group tested all the strains in their collection as well as strains from around the world that were delivered to Peoria via the Navy and Air Force in the form of soil samples. In addition they hired a woman in Peoria to "scour the markets, bakeries, cheese stores, etc". She became known locally as "Mouldy Mary". In the end, in July 1943, the breakthrough strain came from a housewife in Peoria who brought in a mouldy cantaloupe. Whereas Florey's strain produced 2-4 Oxford Units (OU)/ml, the cantaloupe strain (NRRL 1951) yielded 250 OU/ml. It was sent to manufacturing companies in May and June 1944. But the effort did not stop there. NRRL 1951 was sent to Demerec's lab at Cold Spring Harbor where an X-rayed derivative was produced that yielded 450-500 OU/ml. A further UV irradiated derivative, produced at the University of Wisconsin yielded up to 900 OU/ml. When Raper was writing in 1978, he said that he believed that there were then strains producing up to 50,000 OU/ml, but that all those in use in the world, as far as he knew, were derived from the Peoria cantaloupe strain. He was very proud of that. Fleming, Florey and their colleague Chain were award the Nobel Prize in 1944. Five members of the Peoria Penicillin Team, including Kenneth Raper, were awarded the Lasker Award in 1946.

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