Int. J. Dev. Biol. 63: 333-342 (2019)
https://doi.org/10.1387/ijdb.190272vn

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Individual and collective behaviour in cellular slime mould development: contributions of John Bonner (1920-2019)

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ABSTRACT John Bonner used the cellular slime moulds to address issues that lie at the heart of evolutionary and developmental biology. He did so mostly by combining acute observation and a knack for asking the right questions with the methods of classical embryology. The present paper focusses on his contributions to understanding two phenomena that are characteristic of development in general: chemotaxis of single cells to an external attractant, and spatial patterning and proportioning of cell types in the multicellular aggregate. Brief mention is also made of other areas of slime mould biology where he made significant inputs. He saw cellular slime moulds as exemplars of development and worthy of study in their own right. His ideas continue to inspire researchers.

KEY WORDS: *chemotaxis, Dictyostelium, proportioning, sorting out, evo-devo*

Introduction

More than anyone else, John Tyler Bonner (1920-2019) sparked the contemporary interest in the biology of *Dictyostelium discoideum* and other cellular slime moulds (CSMs). He highlighted features of the CSM life cycle that later were picked up by others. His book "The Cellular Slime Molds" (Bonner 1959/1967) long served as the Bible of workers in the field. Obviously, it lacks a great deal of what we know today about CSM biochemistry and molecular biology. Still, the passage of time has not reduced its importance. It has come to be acknowledged as a classic and deserves to be read for the insights it contains. Half a century on, he wrote a second book with the same broad scope (Bonner 2009). It is more up to date but sticks to the cell- and organism-level approach to development and evolution that he favoured. Besides the books, Bonner published a great many papers on the CSMs. The present article, meant for the general reader, not specialist, offers a subjective view of some important problems that he grappled with. There is an occasional remark concerning their present status, but no attempt to be comprehensive or go into details.

The CSMs are free-living amoebae that are common in the soil and animal dung. After exhausting their bacterial food, they cease dividing and undergo a dramatic unicellular-to-multicellular transition. Single cells aggregate, form a cylindrical, polarised multicellular structure (the slug) that moves to the soil surface, and differentiate into a fruiting body that consists of a mass of spores held aloft by a stalk that, in many species, is made of dead amoebae (reviewed in Bonner 1967, Raper 1984, Kessin 2001). Other articles in this special issue of IJDB show that the nature of the life cycle, the ease with which cells can be handled, and similarities with metazoans in their genetic and regulatory organisation have made one CSM, *Dictyostelium discoideum*, the organism of choice for addressing several problems of cell and developmental biology.

Bonner spearheaded the transition of the CSMs from interesting curiosities to ideal objects for studying many of the central problems of biology. His contributions to our understanding of what makes them important emerged in publications that spanned an astonishingly long working life of 75 years. As much as a developmental biologist, Bonner was an evolutionary biologist. Indeed, he was among the leading evolutionary theorists of our time and one of the main forces behind the resurgence of interest in "evo-devo". He carried out his evolutionary pursuits primarily through books. For indications of what they were, see Nanjundiah (2019a), or Nanjundiah (2019b), the second of which illustrates a variant of his hypothesis that in microorganisms, phenotypic evolution is often neutral.

Unless another species is explicitly mentioned, this article deals with observations made on *D. discoideum*. The material is organised into three sections headed Background, Contributions and Overview, with contents that are intended to provide a historical perspective for non-specialists. Accordingly, except for Bonner's works, citations to the wider literature are fragmentary and incomplete. "Background" consists of an extended excerpt from his own recollections of what led him to the slime moulds in the

Abbreviations used in this paper: CSM, cellular slime mould.

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Submitted: 7 August 2019; *Accepted:* 20 August, 2019.

ISSN: Online 1696-3547, Print 0214-6282

first place (Bonner 1991). "Contributions" is sub-divided under three heads: Aggregation and Chemotaxis, Spatial Patterning and Tissue Proportioning, and Other Contributions. The first two sections highlight selected examples of Bonner's work related to CSM development; the third draws attention to the range of his CSM-related work (it would take too long to discuss everything he did). The article ends with an overview. The two appendices contain descriptions, mostly in Bonner's words, of two episodes: Albert Einstein's interest in the slime moulds and the discovery of cyclic AMP as the *D. discoideum* acrasin.

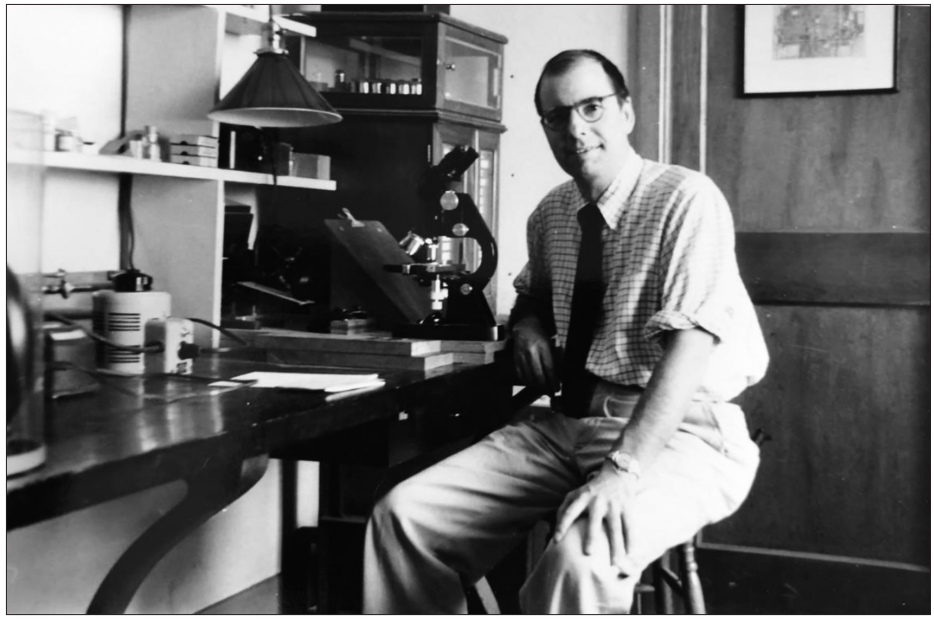

Fig. 1. Bonner in the late 1940s or early 1950s, in his laboratory in Princeton *(photograph courtesy Dr. Rebecca B. Roberts).*

Background

"I first became interested in cellular slime molds when I was an undergraduate at Harvard University in 1940. Beginning in my freshman year I fell under the spell of Professor William H. Weston. He was a cryptogamic botanist and a student of his predecessor, the illustrious Roland Thaxter. 'Cap' Weston was a man of exceptional warmth topped with a wonderful sense of humor. Furthermore, he had a great enthusiasm for experimental studies on lower plants; an enthusiasm that was contagious and I caught the disease. At the same [time] I felt that the subject with the most interesting problems for the future was developmental biology, which then consisted primarily of animal embryology. (In fact the term "developmental biology" was not invented until some years later by Paul Weiss). How could I reconcile these two great passions of my biological youth? The answer, I felt, was to find some lower organism that could be used to study development."

"At first I was greatly tempted to use water molds (phycomycetes) as an experimental organism, but by chance I found in Cap Weston's outer office a copy of Kenneth Raper's Ph. D. thesis which he had done under Weston a few years earlier. I became enormously excited and wrote immediately to Raper, asking him for reprints of his early work and for a culture of *Dictyostelium discoideum*, his newly discovered species which has become so central in all the many studies on slime molds. On the top reprint he wrote "with the hope for your continued interest in these organisms". In later years we became good friends and I used to twit him that perhaps he now regretted his early wish."

"It is surprising to me to think that during the next few years he and I were the only two people working on the cellular slime molds. I can remember during that period, when I gave seminars on my experimental research, the organisms were so unfamiliar to biologists in general that I had difficulty getting beyond a description of the life cycle and on to my experiments. The wonders of the life cycle were quite sufficient to enthrall the audience. The fact that growth occurred first in separate, independent amoebae which, after finishing off the supply of bacterial food, then came together by aggregation to form a well organized multicellular organism was so different from the way all familiar animals and plants developed that my audience seemed not to be able to concentrate on my experiments. After a seminar I gave at the Marine Biological Laboratory in Woods Hole I received a letter from the science reporter at the New York Herald Tribune saying he understood I had done something more important than inventing the atomic bomb; I had created a multicellular organism. I assured him that God had done that and fortunately for me the reporter restrained his journalistic zeal. Jumping from this low point in my early career to a high point, I was asked to come to Yale to give a seminar when I was still a graduate student. After the seminar, which was attended by what seemed a paralyzingly large number of people, Professor Ross G. Harrison, then in his 80's whom I, along with everybody else, considered to be the world's greatest living embryologist, came up to me and said if he were starting all over again, he would work with slime molds. It was a moment for me that made walking on clouds a simple matter and I can feel the glow to this day."

"The early work by both Raper and me, and indeed our later work as well, was primarily biological. I have often asked myself whether or not this was a wise course; would it not have been better if I had followed the new trends by working on the biochemistry of development and, more recently, on the molecular biology of development? I will never know the answer to this question, but I do believe that there is great merit in pursuing development from all three directions, and that the experimental biologist often leads the way by delineating the nature of the problem. He or she is in a better position to see the whole picture and frame the questions that need to be answered." (Bonner 1991).

"It is difficult to believe that it was once necessary to prove that cellular slime molds aggregated by chemotaxis. In the 1940s it was thought that most morphogenetic processes, which often had been assumed to be chemotactic, were probably not so, and could be explained by other mechanisms such as 'contact guidance.' Chemotaxis had first been postulated for cellular slime molds by C. Potts in 1902, but it was not until the earlier work of

Ernest Runyon and this paper that the idea became accepted. My research was done at Harvard after I left the Army to finish my graduate studies; the paper was my Ph D. thesis." (Bonner 1979)

Contributions to chemotaxis and aggregation

A defining feature of the CSMs is that they become multicellular by aggregation, not by divisions of a fertilised egg. In simple yet elegant experiments that went into his PhD thesis, Bonner (1947) demonstrated that the centres towards which *Dictyostelium discoideum* amoebae moved, released an attractant that worked over long distances, plausibly by diffusing from the source. A time-lapse film of development that he had made earlier, as an undergraduate, displayed cells aggregating in oscillatory bursts of centripetal movement (it caused so much excitement that Einstein demanded a personal screening; see Appendix A). L. J. Savage analysed the data and concluded that sensitive amoebae could respond to a relative gradient of as little 2% across the cell length. The attractant induced cellular elongation, orientation and movement; subsequently, the induction of stickiness between cells was added to the list of its effects (Bonner 1944). He named the attractant *acrasin* (after Acrasiales, the name given to the group to which CSMs had been assigned because amoebae retained their identity after aggregating, i.e. did not form a syncytium). Eventually the *D. discoideum* acrasin was identified as cyclic AMP (Konijn *et al.,* 1968, 1972; Bonner *et al.,* 1969; Appendix B carries excerpts from Bonner's remembrance of the event). It was known that not all CSM species used the same chemoattractant for aggregation (Shaffer 1962). Wurster *et al.,* (1976) and Shimomura *et al.,* (1982) discovered that a dipeptide, christened glorin, was the attractant in *Polysphondylium violaceum,* and there are more. It had been thought that the diffusible chemical used by vegetative amoebae of several CSM species to target *E. coli* bacteria could also be cyclic AMP (which the bacteria released) but Pan *et al.,* (1982) found that the food-attractant was folic acid. Bonner was involved in all three pieces of research.

The discovery that cyclic AMP was the long-sought aggregation pheromone in *D. discoideum* led to an outbreak of activity which continues to this day (reviewed in King and Insall 2009). The sequence of events responsible for chemotaxis, the relaying of acrasin from cell to cell and its degradation – both of which had been deduced by Shaffer (1962) - and the oscillatory production and release of the signal remain topics of intense investigation (Halloy *et al.,* 1998). We do not understand the chemotaxis by feeding amoebae to folic acid in comparable detail, and the same holds for other acrasins. Initially Bonner (1947) had ruled out a role for contact-mediated guidance in aggregation, because chemoattraction worked across an underwater gap. It seems, though, that intercellular contacts may guide cell movements at a later stage of morphogenesis in *D. discoideum* (Fujimori *et al.,* 2019).

An early study showed the presence of acrasin in multicellular stages. In the slug, its concentration was highest at the tip (Bonner 1949), the region that contains presumptive stalk cells, as will be discussed in the next section. The demonstration that high cyclic AMP could induce the differentiation of stalk cells, seemed to confirm the link between position and cell fate (Bonner 1970). The full picture is more complex. At physiological levels, cyclic AMP induces spore differentiation (Kay 1982). The stalk effect seen by Bonner may have been a secondary consequence following the production of a chlorinated hexanone, differentiation-inducing factor (DIF), that induces the differentiation of a genetically-defined subset of stalk cells while inhibiting spore differentiation (Kay *et al.,* 2001). It transpires that the cyclic AMP has roles in the multicellular stage of several CSMs that may, but need not, be related to its status as the agent of intercellular communication during aggregation (Schaap *et al.,* 2006; Singer *et al.,* 2019).

Bonner's researches uncovered other aspects of the aggregation process that are not understood in detail. In several CSM species the size of an aggregation territory, or the spacing between fruiting bodies, barely changes over a huge range of cell densities (Bonner and Dodd 1962). A possible explanation is a gaseous inhibitor that prevents one centre from forming close to another (Bonner and Hoffmann 1963) and leads to distance-dependent competition between potential centres (Waddell 1982). In *D. mucoroides* the inhibitor is ammonia, and it works opposite to cyclic AMP, which functions as an activator (Thadani *et al.,* 1977). Ammonia and cyclic AMP function similarly in *D. discoideum* too, and that might explain the roughly uniform spatial distribution of centres (Feit and Sollitto 1987).

Two findings related to chemotaxis remain to be explored further. Keating and Bonner (1977) found, as Samuel (1961) had earlier, that amoebae of *D. discoideum* repelled one another, whereas those of *P. violaceum* did not (from the description, the amoebae may have been in a vegetative state). Cone and Bonner (1980) reported that the calcium ionophore A23187 induces aggregation centre formation in *P. violaceum.* In *P. violaceum*, the probability that a starved cell produces the chemoattractant, which is zero to begin with, rises very slowly with time. This is unlike what is seen in *D. discoideum*, where the

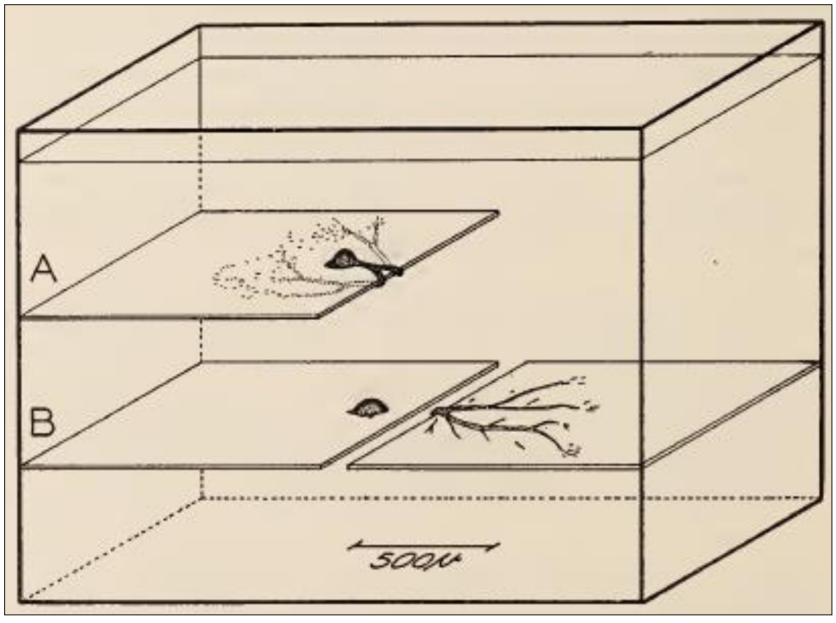

Fig. 2. Chemotaxis of cellular slime mould amoebae towards a diffusible substance (the cells are under water). (A) *Amoebae below a coverslip are attracted to a centre that is above it.* **(B)** *Amoebae attracted across a gap (from Bonner 1947).*

corresponding probability rises more rapidly, so that if a centre of aggregation is removed, another replaces it at once. Thus, for all practical purposes, the *P. violaceum* centre is a specialised cell that differentiates early in a very small fraction of the population (Shaffer 1962). The ionophore effect raises the interesting question whether calcium regulates the dynamics of centre formation, that is, influences the developmental transition required for a starved cell to become capable of spontaneously releasing the chemoattractant.

Post-aggregation differentiation and patterning in the slug

The cells in the fruiting body can be members of the same clone. Much before they are visibly differentiated, they differ in presumptive fates, as shown by the fact that they are segregated functionally along the long axis of the slug; on the whole, presumptive stalk cells occupy the front and presumptive spore cells, the back. The anterior-most region, the slug tip, has special properties that make it resemble the Spemann organiser (Raper 1940). Some cells within the posterior region are 'anterior-like' in that they resemble presumptive stalk cells (Bonner *et al.,* 1955). They play an important role during culmination and tip regeneration (Sternfeld and David, 1982; but see Mohri *et al.,* 2019). What causes differences to arise among a seemingly homogeneous population of cells? And what leads to their spatial separation along the long axis of the slug?

Bonner (1944) claimed initially that that the aggregate centre became the slug tip, the amoebae which joined earliest became pre-stalk cells and the amoebae which joined later became presumptive spore cells. The implication was the temporal order in which cells entered the aggregate determined their spatial distribution along the anterior-posterior axis of the slug and so their differentiated fate. His opinion changed when he realised that pre-aggregation amoebae, even when they were members of a clone and were raised in the same environment, could differ; and those differences could predispose the appearance of functional differences between post-aggregation cells (Bonner 1957). Bonner called it "range variation" (Bonner 1965). The initial hint came from the observation that aggregating cells varied in their speed of movement; within the migrating slug, the relatively faster cells accumulated in the front (Bonner 1952). Cell size was another aspect of pre-aggregation heterogeneity; larger cells tended to occupy the slug anterior and became presumptive stalk cells (Bonner and Frascella, 1953; Bonner *et al.,* 1955). Functional heterogeneities could also be imposed on pre-aggregation amoebae by mixing genetically or otherwise distinguishable cells, which drove home the fact that cells could sort out after aggregation in accordance with their fates (Bonner 1959; Bonner *et al.,* 1971). Sorting out can occur through either differential adhesion (Tasaka and Takeuchi 1979) or differential chemotaxis to cyclic AMP (Matsukama and Dusrston 1979) or, as Bonner advocated, different speeds of movement, or by a combination of several mechanisms.

Bonner's work pointed to a significant distinction between the effects of heterogeneities in genotype and phenotype. Genotype differences among pre-aggregation amoebae could bias cell-type differentiation by influencing their phenotypes. However, the differences were not essential, in the sense that they were not necessary for normal development. On the other hand, even among members of a clone, differences in phenotype were always present and, it seemed, could be all-important (Bonner 1963a); Takeuchi (1963) drew the same inference independently.

Even at the time, it was obvious that there was more to it. Raper (1940) had cut slugs transversely and thereby changed the position of a cell from relatively anterior to relatively posterior, or vice-versa. Based on the expected fates of cells along the anterior-posterior axis of the unfragmented slug, a cut fragment could consist overwhelmingly, if not entirely, of presumptive stalk cells or presumptive spore cells. Raper found that without any growth or increase in cell number, both fragments gave rise to normally proportioned fruiting bodies (in the case of the anterior fragment, only after a lapse of time). Thus, the fate of a cell could switch from presumptive stalk to presumptive spore, or the other way around. Cell type-specific markers confirmed that the spatial pattern could regulate – in other words, that whatever pre-aggregation differences were present, could be overridden; this meant that there was a second layer of control for cell differentiation (Bonner 1952; Bonner *et al.,* 1955). That layer had to operate via intercellular interactions that took place among cells that differed a lot, very little, or, conceivably (within the limits of experimental detection), not at all.

To what extent can intercellular differences be reduced without affecting normal development? In a remarkable experiment designed to test this, Bonner *et al.,* (1985) took cells of *D. mucoroides var. stoloniferum* through six asexual life cycles - from spores to amoebae to aggregates to fruiting bodies – without a feeding phase, and therefore cell growth or division, intervening. Relative cell-to-cell differences remained high all through. (Visual inspection of figure 5 of Bonner *et al.,* (1985) indicates that in going from the parental to the fourth life cycle, there is an approximately four-fold decrease in spore volume and five-fold decrease in its standard deviation, so that the relative variation in spore sizes remains more or less what it was at the start.)

Therefore, as in embryonic development, it is not possible to think of mosaicism, which can lead to sorting out between cells with prior tendencies (their positions in the embryo being decided thereby), or regulative development, which can lead to differences arising via intercellular communication among potentially equivalent cells at different locations (their fates being decided thereby), as mutually exclusive alternatives. Which mode operates in a given situation, depends on the circumstances (Bonner 1963a, 1992). A number of pre-aggregation differences are known to bias cell fate, but it remains an open question whether normal development can proceed in the absence of any differences whatsoever (reviewed in Nanjundiah and Saran 1992).

Proportioning

"The supreme problem in the differentiation of the cellular slime molds is that of proportions." (Bonner 1967, p.150). Harper and Raper had remarked that in several CSM species, the relative proportions of the stalk and sorus (spore mass) appeared to be the same in differently sized fruiting bodies (Raper 1935). As discussed in the previous section, it is characteristic of regulative development that tissue proportions accommodate to the size of the individual. This is true of CSMs too, though as the fruiting body size decreases below ~100 cells, there is a systematic bias in favour of the stalk pathway (Nanjundiah and Bhogle 1995). The current world record is held by a fruiting body of *P. pallidum* (now *Heterostelium pallidum*) with 4 spore and 3 stalk cells (Bonner and Dodd 1962). Fig. 3, left, shows that CSM fruiting bodies with greatly differing proportions

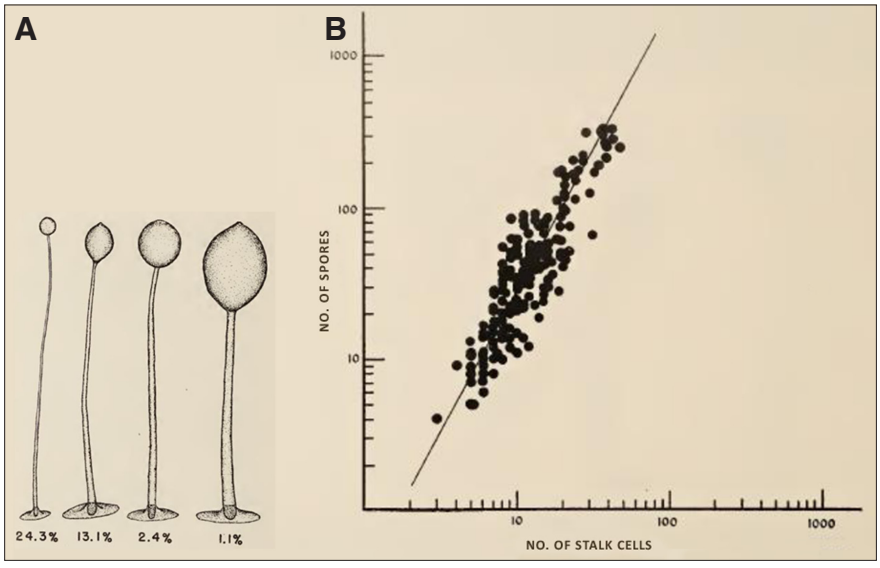

Fig. 3. Tissue proportioning in the cellular slime moulds. (A) *An indication of the range over which the percentage of stalk cells varies between individual fruiting bodies of* D. discoideum *of approximately equal height.* **(B)** *Double logarithmic plot of spore cell versus stalk cell numbers in fruiting bodies of* D. purpureum, D. mucoroides *and* P. pallidum *(combined data). The fruiting bodies in the left panel are ~1mm tall. The panels are from Bonner and Slifkin (1949) and Bonner and Dodd (1962), respectively, and are reproduced with permission.*

do occur but the mean is more or less invariant (Fig. 3, right). The precise description of proportioning occupied Bonner all his life. He and his colleagues carried out quantitative measurements of spore and stalk tissue sizes (estimated variously in terms of dry weight, volume or cell number; Bonner and Eldredge 1945, Bonner and Slifkin 1949, Bonner 1957, Bonner and Dodd 1962).

They discovered that proportioning held over sizes ranging over at least three orders of magnitude (as mentioned, there is a slight fall in spore proportions as the fruiting body size declines, as has been seen in *D. mucoroides* and *D. discoideum*). In *D. discoideum* and *D. mucoroides* (as well as *D. purpureum*), but not in *P. violaceum or P. pallidum/Heterostelium pallidum*, presumptive stalk and spore cells could be identified in the migrating slug; their numbers, or volumes, were in roughly the same proportions as in differentiated stalk and spore cells in the fruiting body (Bonner and Slifkin 1949; Bonner *et al.,* 1955; MacWilliams and Bonner 1979). This presents an additional puzzle in the case of species such as *D. mucoroides*, in which, as the slug migrates, there is a continuous conversion of presumptive stalk cells to terminally differentiated stalk.

Bonner (1957) put together observations on pre-aggregation heterogeneity and regulation and came up with a theoretical model that was to be revived by others in different guises. Its essence was that (i) to begin with, the cells that join an aggregate are heterogeneous; (ii) their speeds of movement differ, because of which (relatively) fast cells - presumptive stalk cells - end up in the front of the slug and relatively slow cells in the back; (iii) a factor is supplied by slow cells to fast cells at a rate proportional to the number of slow cells, (iii) the factor is utilised by fast cells at a rate proportional to their own number, (iv) the factor is used by presumptive stalk cells to differentiate terminally into stalk cells and (v) cells that do not become stalk, become spores. The upshot is that cell type proportions equal the ratios of the rates at which the relevant processes occur. At one stroke Bonner combined initial heterogeneity (which could result in sorting-out) and intercellular communication (which could give rise to regulation) in a unified scheme for explaining spatial pattern and tissue proportions. Not just that, he pointed out that differences in the times of onset and durations of the various processes could account for differences in cell type patterns between the slugs of *D. discoideum*, *D. mucoroides* and *Polysphondylium*.

Subsequently Bonner discovered that it was possible to study spatial patterning in artificially generated 1-dimensional and 2-dimensional "aggregates" of *D. discoideum* (the geometry makes it impossible for morphogenesis to proceed further; Bonner *et al.,* 1995, 1998; Bonner 1998; Bonner *et al.,* 1999). This opened up the opportunity for a combined experimental and theoretical approach to the study of spatial patterning that remains to be grasped. The 1- and 2-dimensional cell groups mimicked normal 3-dimensional slugs but there were interesting differences. Oxygen had been found to cause patterning without morphogenesis in underwater clumps (Sternfeld and Bonner, 1977), and in a 1-dimensional array, an oxygen gradient led to presumptive stalk cells to differentiate within a few minutes. That may be more reflective of oxygen starvation than normal development (as a referee pointed out). What is noteworthy is that such a simple geometry has been demonstrated to be achievable; it makes the possible drawback less serious. By careful adjustment of environmental conditions, for instance in a microfluidic setup, it should be possible to study pattern formation in other one-dimensional configurations. A linear or filamentous Dictyostelium is a wonderful prospect. Two-dimensional slugs threw up the surprising finding that the tip was a geometrical region at the anterior rather than a fixed group of cells.

With the help of an ingenious scheme based on energetics, Trenchard (2019) has recently revived the idea that sorting out among cells with different speeds can explain spatial segregation in the slug. There have been many variants of Bonner's model for cell type proportioning (MacWilliams *et al.,* 1985; Nanjundiah and Bhogle 1995; Söderbom and Loomis 1998; Kay *et al.,* 2001), some of them with suggestions regarding the nature of the processes and feedbacks that may be involved. One- and two-dimensional slugs are beginning to be studied in microfluidic setups; so far, less with the aim of throwing light on what happens during normal development than for making use of *D. discoideum* to generate interesting group dynamics (Gholami *et al.,* 2015, Eckstein *et al.,* 2018).

Other contributions

Because of constraints on space and the focus on CSM development, this survey has touched on only a part of Bonner's work. Topics left out include studies on slug movement, especially the finding that speed goes up with size (Bonner *et al.,* 1953; Bonner 1995); phototaxis and thermotaxis in the slug (Bonner *et al.,* 1950); and the role of ammonia in mediating positive and negative taxes of the slug and fruiting body (Bonner *et al.,* 1986; Bonner *et al.,* 1989). However, some mention must be made regarding a ques-

tion that engaged him from the beginning, namely the evolution of development, a theme that he tackled in many of his books including the two that dealt with CSMs (Bonner 1967, 2009).

Bonner wrote two papers devoted to CSM evolution (Bonner 1982; Bonner 2015) and collaborated on another that pointed out common principles underlying social behaviour in the CSMs and social insects that hinted at convergent evolution of an unexpected sort (Gadagkar and Bonner 1994). He speculated on how multicellularity might have evolved in a CSM ancestor, how diverse patterns of development might have emerged in different groups, and what might have led to the evolution of a stalk composed of dead cells. Regarding the origin of aggregative multicellularity itself, he stressed the importance of the morphology of the fruiting body for efficient spore dispersal. Later he veered towards the notion, increasingly prevalent among plant and animal ecologists, that stochastic factors, rather than selective forces, may have contributed to the pattern of geographical diversity shown by CSMs (Bonner 2009, p.37).

When it came to the apparent paradox of why stalk cells died "for the sake" of other cells, namely the spores, he felt (and stated in personal exchanges) that stalk cell death was an example of altruism at the genetic level. That was because stalk and spore cells were likely to share genes – or, if they were members of a clone, have the same genes. Therefore, the evolutionary origins of stalk cell death had to be investigated within the scope of Hamilton's concept of inclusive fitness, not by assigning a group-level component of fitness to the fruiting body. He thought that unlike between-colony selection in social insects, the strength of selection between fruiting bodies was not likely to be significant. At the same time, he noted the importance of two potentially complicating factors. First, there was abundant genetic, and more so phenotypic, variation among the members of CSM groups in the wild. That raised the possibility that chimaeras were not uncommon, and reduced the scope for cooperation (Bonner 2009). Second, as some studies had shown, co-occurring low-fitness strains were capable of phenotypic complementation, which too may have played in the evolution of CSM social behaviour (Bonner 1967, p.167-168). And importantly, the role of ecological factors in promoting development, as, for instance, illustrated by the synergistic effect of the fungus *Mucor* on *D. mucoroides* (Ellison and Buss, 1983), was largely unknown (Bonner 2009).

As for the diversification in patterns of development in the CSMs, he believed that heterochrony, i.e. temporal differences in the times of action of regulatory genes, was the key (Bonner 1982). Along with that, he thought that selection for increased size facilitated the evolution of division of labour (in the case of the CSMs, differentiation into presumptive stalk and spore); this was the central element in his hypothesis for the evolution of multicellularity in general (Bonner 2006). Developmental plasticity was an important feature. In small aggregates of *D. lacteum* (now *Tieghemostelium lacteum;* Sheikh *et al*., 2018) every cell differentiated into a spore and the stalk was an extracellular product, whereas large aggregates displayed division of labour in the form of non-reproductive stalk cells (he pointed out that the volvocine alga *Eudorina* provided a parallel example; Bonner 2003). One sees that he did not confine himself to a unique explanation for the evolution of social behaviour in the CSMs. When it came to the evolution of developmental, especially morphological, differences between CSM species, Bonner, like everyone else, was led astray: it turns out that the traditionally assumed relatedness between species based on morphological similarity does not reflect phylogenetic relationships (Schaap *et al.,* 2006, Sheikh *et al*., 2018). As we are learning from DNA-based phylogenies in several groups, the reason is that grades of organisational complexity need not reflect clades of nearest relatives.

Overview

The major themes that engaged Bonner were cell and tissue movement (in particular, directed movement), the spatial distribution of cell types (i.e. pattern formation), tissue proportioning, and differentiation. Because it was the disappearance of food that set off the CSM developmental sequence, the evolutionary driver behind the life cycle had to be selection for dispersal; it was the trigger behind aggregation, movement to the soil surface, spore formation and fruiting body formation. He stressed that the proper way to look at the evolution of organisms was in terms of the evolution of life cycles. In the CSMs too, each "part" of development, each adaptation, had to be viewed as part of a life cycle that had to be understood as a whole; not, as is often the case, in terms of a set of frozen snapshot phenotypes with their own evolutionary trajectories. Genes whose functioning could alter the temporal course of developmental events were crucial because in their case, natural selection could potentiate qualitative changes in developmental sequences. There were two respects in which he thought one had to take note of organising principles beyond the gene level. One was that cell and tissue interactions could give rise to drastically different outcomes when an environmental parameter was tweaked. The most familiar example is the scheme proposed by Turing: in principle, mutual interactions between just two diffusing chemicals can give rise to patterns that include spots, stripes and travelling waves (Turing 1952). His colleagues McNally and Cox (1989) made a plausible case for a Turing-like mechanism for the pattern in whorls of *P. pallidum* (now *Heterostelium pallidum*) fruiting bodies. Because of those reasons, he favoured Newman's proposal that a genetic change could follow, rather than precede, a change in phenotype; genes acting more as stabilisers than innovators, so to speak (Newman 1992; Bonner 1993, p.89).

For someone who had always expressed himself as a selectionist, he advanced a bold hypothesis late in life (Bonner 2005). Typically, it was in a book and also typically, he had dropped hints of it before. The hypothesis was that the force of natural selection was stronger in large, complex organisms (whose passage from zygote to adult involved several interlocked developmental stages) than in small, simple ones (such as protozoans, or even in the CSMs). Consequently, the latter had the possibility of evolving neutrally from one morph to another. His final paper (Bonner 2019) has been published posthumously. It deals with a related theme and makes the point that many simple organisms stay that way because they can do without obligatory sexual reproduction. Both hypotheses are radical and will no doubt be examined over years to come. A beginning has been made by Hamant *et al.,* (2019), who try to link polyclonal development (as is possible in the CSMs), small size, and a dependence on external resources, to asexual reproduction; and clonal development (as in metazoans), large size and an assurance of internal resources, to sexual reproduction.

Bonner's studies on CSM development mostly involved a single species, *Dictyostelium discoideum*. In choosing which phenomena to focus on, he was guided by the prior work of Kenneth Raper,

especially four fundamental papers (Raper 1935, 1940, 1941; Raper and Thom 1941; see "Background"). Building on them, he carried out quantitative measurements that identified regularities in developmental processes. Always he related his findings to the big conceptual issues that dominated developmental biology, or embryology, for much of the 20th century, and he did so from an evolutionary standpoint. He was ingenious in identifying analogies to the slime mould way of life. The range of organisms from which he drew them was astonishing. It went along with the conviction, expressed early in his career, that there was much to be learnt in biology from analogical reasoning and functional approaches (Bonner 1963b). One can sense his constant searching to accommodate that stand to a neo-Darwinian attitude. He saw himself in the tradition of Driesch, Conklin, Spemann and Harrison, with the advantage that he worked with a much simpler system. As in their case, inferences were drawn from careful observations carried out with little more than a microscope, vital dyes and minimal experimental manipulation. In turn, his findings motivated, and continue to motivate, the search for mechanistic explanations based on biochemistry and molecular biology – of which he made little use, except in the course of occasional collaborations. Our ignorance about most living organisms is vast, all the more so when it comes to microorganisms that live in groups, including (quite possibly) many as-yet undiscovered CSM species. Given that, it is clear that Bonner's approach of careful observations of morphology and behaviour at the cell and group level, and formulating the right questions to ask, will continue to be important. And, as he kept insisting, cellular slime moulds deserve to be studied for their own sake, not just as models for something else (Bonner 1999).

Acknowledgements

I thank Dr. Rebecca B. Roberts for supplying the photograph of John Bonner and John Wiley and Sons for permission to reproduce figures 1 and 3. Stuart Newman, Kei Inouye, Albert Goldbeter, Rob Kay and an anonymous referee drew my attention to portions that were unclear and made several useful suggestions, for which I am grateful. The hospitality of the International Centre for Theoretical Sciences, where much of the work in this paper was carried out while visiting as an Associate, is acknowledged.

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Appendix A: Albert Einstein is intrigued by the slime moulds

John Bonner studied for a B.Sc. degree at Harvard. He obtained it, *magna cum laude* with highest honours in biology, in 1941; the film he showed Einstein was part of his Senior thesis. After spending time in the Army during the War he completed a Ph.D. in 1947, also from Harvard (under Weston, who had supervised Kenneth Raper's PhD thesis research some years before) and joined Princeton as Assistant Professor the same year. Much earlier, in 1929, Kurt Noack and Arthur Arndt had co-authored a high-quality time-lapse film of the development of *D. mucoroides*. It clearly shows oriented movements and oscillatory aggregation. Some thought that the oscillations were no more than the jerkiness that is a concomitant of the time-lapse technique, a familiar experience from old silent films. The paper that described the observations appeared in 1937, after Arndt's death (Arndt, A. (1937). *Rhizopodenstudien III. Untersuchungen über Dictyostelium mucoroides Brefeld*; Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen 136 (5): 681–744). The Noack-Arndt film should be viewable (http://www.filmarchives-online.eu/viewDetailForm?FilmworkID=358af36868fa2a8548b03bb2c2cbbf2a); Bonner's certainly is (https://www.youtube.com/watch?v=bkVhLJLG7ug). The Einstein story is in Bonner's words.

"Shortly after I arrived in Princeton we were invited to a cocktail party and I met Paul Oppenheim, a charming German philosopher of science who told me he was very interested in biology and asked me what I worked on. He pressed me even though I told him I worked on very curious amoebae, and when I told him they were slime molds his face lit up and he began to tell me all about their peculiarities. I was stunned and asked him how he knew this because at that time even most biologists did not know of their existence. He said that some years ago in Germany in the 1930s he had heard a lecture by Arthur Arndt, who showed a wonderful time-lapse film of their development (and it is indeed a fascinating film; I still have a copy). He went on to say that the lecture was quite extraordinary because Arndt said that the life cycle of these slime molds was so amazing that any materialistic explanation was out of the question; it could only be explained by some mystical vital force. There is no hint of this in Arndt's 1937 paper on the organism, but he apparently felt no constraints in a lecture.

"After that Paul Oppenheim and I became friends, he always asking me about my experiments and at the same time trying hard to make a philosopher, a logical positivist, out of me. He was a close friend of Albert Einstein and that was the main reason he eventually chose Princeton after he had to leave Germany. One day he called me up and said that Professor Einstein would like to see my film (my old senior thesis film) that he had told Einstein about, and would I come to Einstein's house to show it." (From Bonner 2011, *Lives of a Biologist*, p.96-97). The story continues: "I was obviously thrilled and showed up with film and projector at the appointed hour. We had trouble finding a suitable screen but finally took a wall map of the United States and turned it around. After the viewing, Professor Einstein asked me if I would come into his study to discuss what we had seen. We were joined by the mutual friend who had arranged the meeting and Miss Dukas, Professor Einstein's secretary. We talked for some time, and what impressed me particularly was the depth of his questions. He wanted to know immediately the answers to all those questions that have been pursuing me all my life: How was the life cycle controlled so that it was the same each generation? Why does this kind of organism exist at all? Where does it fit in with other animals and plants? And he had many related questions. I wish I could have another conversation with him now; I have had much more time to think about these problems." (Bonner, 1993: *Life Cycles*, p.66).

The 1984 BBC TV film, "Professor Bonner and the slime molds" (http://www.tv.com/shows/horizon/professor-bonner-and-the-slime-molds-1261548/) shows Bonner at his best as he explains what makes these organisms so fascinating. He said it brought him fame in the village in Canada where he vacationed every year; till then he had been one more nondescript summer regular (see Appendix B).

Appendix B: the long-sought acrasin turns out to be cyclic AMP

"The other big event in my lab happened a couple of years later. Ever since 1959 we have spent the summers in Cape Breton, Nova Scotia, first in a cabin and then in a small house on the Margaree River. One morning in the summer of 1967 my work was interrupted by an excited telephone all from my laboratory in Princeton: David Barkley, a graduate student, and Theo Konijn, a visiting colleague from Holland, had made a big discovery. They were both on the line and they were talking so rapidly that I had to make them repeat everything twice so I could understand it.

"As I have mentioned earlier, in my Ph.D. thesis research I showed that slime mold amoebae are undoubtedly attracted by a chemical substance when they come together to. form a multicellular slug. For twenty years various workers had made attempts to find what was the chemical nature of the attractant, which I had called "acrasin". For a while it looked as though it might be a steroid hormone because a worker in the field had showed that urine of a pregnant woman had the ability to attract amoebae. Some years later a young assistant and I tried to repeat this finding using the urine from someone who was not pregnant, and that worked also. [The story was not quite that simple. She used a diluted sample of her own urine, and it worked. I pointed out to her that we had shown that the urine need not be from a pregnant person, and she blushed beet red, and said she had been meaning to tell me that she was going to have a baby. So we tried my urine and it worked too, and I was quite sure I was nor pregnant.] Theo Konijn had done some work that paralleled ours, and he got a grant to come to Princeton so that we could combine forces and attack the problem. He came shortly before we left for Canada to begin his year, and he and David Barkley were having some maple walnut ice cream discussing all that was known of the properties of acrasin. It was a small molecule, given off by bacteria, found in urine. and there were a number of other clues. David said that it had many of the properties of a new substance called cyclic AMP, isolated recently by Earl Sutherland at Vanderbilt University, which he had just learned about in a biochemistry course. […]

"So David and Theo obtained some cyclic AMP and quickly found that it was a strong Amoeba attractant even at exceedingly low concentrations. For slime molds it was a primary messenger as well as a secondary one That is what they were telling me on the telephone. I knew they had hit the jackpot, but I was at a big disadvantage, up in my northern isolation, because I had never heard of cyclic AMP! Their excitement was contagious; I could not continue my work; […].

"It has always seemed to me a bit of extraordinary luck that the solution to the chemical nature of acrasin should have come so easily. Theo Konijn came for the year to work on a problem that he solved in his first month. The result of this work was an enormous interest in many laboratories on how the cyclic AMP oriented cells, and today we have large amounts of biochemical and molecular information on the details" (Bonner, *Lives of a Biologist*, pp137-139)."

Remarkably, for a while the test that Konijn devised for quantitating the chemotactic response of *D. discoideum* amoebae proved to be the most accurate way of measuring cyclic AMP concentrations within the appropriate range. In his hands, it was capable of yielding results to within two-fold accuracy, which beat the binding protein-based assay then available. (Konijn, T. M. (1970). *Microbiological assay of cyclic 3',5'-AMP*. Experientia. 26(4): 367-369.)

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

YelA, a putative *Dictyostelium* translational regulator, acts as antagonist of DIF-1 signaling to control cell-type proportioning
Yoko Yamada, Chris Sugden and Jeffrey G. Williams
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***Dictyostelium discoideum* Sir2D modulates cell-type specific gene expression and is involved in autophagy**
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