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BROWNIAN MOVEMENT IN CLARKIA POLLEN: A REPRISE OF THE FIRST OBSERVATIONS

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INTRODUCTION

Much interest has focussed on Brown's first observations of Brownian Movement* in 1827. His discovery of the phenomenon has been widely misunderstood. It has been believed that Brown's attention was directed to movement of pollen grains themselves (1), and there have been claims (2) that his microscope was not sufficiently developed for the observation of so diminutive a phenomenon. It has become plain that many workers have discussed the problem without consulting Brown's original words on the subject, and it is abundantly clear that the optical propensities of the original microscope(s) owned by Brown have been widely ignored. A recreation of Brown's pioneering demonstration is presented in correlation with the privately printed account Brown himself prepared in 1827.

ROBERT BROWN, BOTANIST

Robert Brown's father James was a Scottish Episcopalian minister with a strong and independent mind. It is clear that his son (born in Montrose, Scotland, on December 21, 1773) inherited a similar intellectual strength, though he did not acquire the father's unbending Christian dogmatism. The young Robert was educated at the Marischal College, Aberdeen, and studied medicine at the University of Edinburgh. By the age of twenty-one he had joined the Fifeshire Regiment of Fencibles as Ensign and Surgeon's Mate. It was then 1795, and the regiment was soon posted to Ireland. He used much of the time to study. Before breakfast he was accustomed to study German grammar, and after the meal he would work on botanical documents until lunch-time. From 1.00pm to 3.00 pm he would see patients. In the evening, if he was not socialising or out to dine, he would continue his scientific work until midnight. He clearly enjoyed long night-time discussions: an entry in his diary notes that he drank "about a pint" of port one evening over a social meal.

In October 1798, the young officer was in London to recruit for the regiment. He was introduced to the eminent botanist Sir Joseph Banks as "a Scotchman, fit to pursue an object with constance and [a] cold mind." Within two years, Banks was planning an epic voyage of discovery to the new territories that we now know as Australia. His natural choice for botanist on board was the young Brown. He sailed under Captain Matthew Flinders at a salary of £420, at the time a generous sum. When they sailed on board the *Investigator* on July 18, 1801, both Robert Brown and Matthew Flinders were twenty-seven. Also on board was an artist and draughtsman of immense talent: the forty-one year old Ferdinand Bauer (brother of the equally renowned Francis).

*NOTE: Brown's usual term was 'movement', and as I have pointed out elsewhere, this is the term recognised in the standard texts (3). In recent years the alternative usage "Brownian Motion" has gained more general acceptance among physicists.

On December 8, 1801 they arrived on the territories of New Holland (as Australia was then called by Europeans). Their point of arrival was King George Sound, on the south-western corner of the great sub-continent. Within three weeks he had collected more than 500 species of plants, almost all of them unknown to western science. Later he stayed for three months at Port Jackson, and ten months more on the island of Tasmania. They returned home to England in October 1805 with vast collections of drawings and notes and many zoological specimens, and with nearly 4,000 different species of plants. Brown was offered a government salary whilst he worked on the material, and devoted the next five years to describing 2,200 of the species, over 1,700 of which were previously unknown. Brown himself nominated 140 new genera. From 1806-1822 Robert Brown served as "Clerk, Librarian and

Housekeeper" to the Linnean Society of London, and he took on Banks's home and collections in Soho Square when Sir Joseph died on June 19, 1820. The stipulation of the bequest was that the collections would be in the care of Brown during his lifetime, and would pass to the new British Museum on Brown's death. But matters moved more quickly, for Robert Brown was able to negotiate the transfer of the specimens in 1827, on condition that they become a permanent part of the British Museum and that he remained their curator for life. This gesture was an important event in the establishment of the great London collections which have since become so important in the world of taxonomy.

Robert Brown had been elected to Fellowship of the Royal Society in 1810, and became a Fellow of the Linnean Society in 1822. He was the President of the Linnean from 1849 to 1853. He died in London on June 10, 1858, just a week before Darwin received Wallace's paper on the theory of "survival of the fittest", and the date of Brown's death ultimately led to the availability of a free date at which Darwin might present his own epoch-making lecture on the theory of evolution to the Linnean Society.

THE OBSERVATIONS OF ROBERT BROWN

Brown used microscopes throughout his adult life. He was an accomplished technician and an extraordinarily gifted observer of microscopic phenomena. Thus, it was Brown who identified the naked ovule in the gymnospermae. This is a difficult observation to make with a modern instrument even with the benefit of hindsight. To Brown this task was immeasurably greater. Typically, he tucked the report of his observation away in a more lengthy publication (4). To a paper by Captain P. P. King, Brown added the following words:

"It would entirely remove the doubts that may exist respecting the point of impregnation, if cases could be produced where the ovarium was either altogether wanting, or so imperfectly formed, that the ovulum itself became directly exposed to the action of the pollen ... such, I believe, is the real explanation of the structure of the Cycadeae, the Coniferae, of *Ephedra,* and even of *Gnetum."*

But it was with the observation of the incessant agitation of minute suspended particles that Brown's name became inextricably linked. The effect, since known as Brownian Movement, was first noticed by him in 1827. His own description of his discovery reveals that he was planning to continue his work on the mechanisms of fertilization in flowering plants, which he had published early in 1826 (5). Having worked on the ovum, it was natural to direct attention to the structure of pollen and its interrelationship with the pistil. The first species to which he turned his attention in June 1827 was the American species *Clarkia pulchella,* [the genus being spelled *Clarckia* by Brown in his account]. He looked with particular care at the structure of the pollen-grains. These he took, not from opened or dehiscent anthers, but from fully-formed pollen sacs that were yet to open and which he dissected at the bench.

He suspended some of the pollen grains in water and examined them closely, only to see them "filled with particles" that were "very evidently in motion". He was soon satisfied that the movement "arose neither from currents in the fluid, nor from its gradual evaporation, but belonged to the particle itself". In due course he was to carry out careful experiments to disprove these alternative explanations, and it has been shown that Brown was able to anticipate the later objections of those who would doubt his capacity to have observed what he claimed (6). It must have been tempting for Robert Brown to assume, as had other workers before him, that here was the very essence of life. Within the germinal cells of living organisms he could perceive movement without end. To a modern eye, well versed in the drama of scientific discovery as the twentieth century comes to its close, this is the immediate explanation. Most of the lay people who have seen the phenomenon conclude that they are watching life itself at work.

It is to Brown's great credit that he was not so easily persuaded. Having seen the phenomenon in a host of living plant specimens he was led to ask whether it persisted in plants that were dead. As he was passing the proofs of his paper for the press, he wrote that he had seen the same phenomenon within pollen grains preserved for about eleven months in an alcoholic solution:

".. particularly of *Viola tricolor, Zizania aquatica* and *Zea mays."* (7)

Robert Brown had read the accounts of many of the earlier workers who had seen this phenomenon, and noted that they tended to associate it with organic matter (on the assumption that is was linked with the mechanisms of life). He writes that it had been assumed they were:

".. elementary molecules of organic bodies, first so considered by Buffon and Needham, then by Wrisberg with greater precision, soon after and still more particularly by Muller, and, very recently, by Dr. Milne Edwards, who has revived the doctrine and supported it with much interesting detail." (8)

Brown moved on to consider a host of clearly non-living specimens, including rocks "of all ages" which yielded the particles "in abundance". In short, he concluded, any solid mineral would reveal the phenomenon subject to its being reduced to a sufficiently fine powdery form. He showed an admirable objectivity in taking up a topic well known to previous microscopists, yet in setting out a revolutionary explanation for its physical (as opposed to its biological or organic) nature.

HOW BROWN OBSERVED BROWNIAN MOVEMENT

Interest in the effect continued unabated. The analysis of Brownian movement by A. Einstein in 1905 led to the formulation of the Boltzmann Constant, and shortly afterwards J. B. Perrin began his publications in Paris. They were later summarized in an extended paper which was later published in English translation as a book in its own right (9). Not only did his account range across the many other workers who published since the time of Brown, but he also demonstrated the effect by projection microscopy. To this day the nature of Brownian movement is debated, and its cause still argued; the mathematics of the phenomenon continues to occupy a significant tranche of contemporary publications in physics. A search through *Current Contents* for 'Brownian

Motion' will often provide several citations each month in the modern literature.

But the central controversy remains: could Robert Brown have observed the phenomenon through his microscope? Was it possible that some alternative effect, some form of 'pseudo-Brownian motion', was responsible for his reports? It was decided to undertake a recreation of his original experiments in order to resolve the matter once and for all. Brownian movement is familiar to all microscopists. The agitation caused by evaporation currents induced by thermodynamic turbulence are well known to all experienced research workers. Brownian movement has such unmistakable characteristics. Its kinetic force is directly related to particle size, and the vector of the force that gives rise to the movement is not in any way consistent, nor does it result in motion in a specific direction. To the eye of the microscopist it is, instantly and unmistakably, Brownian movement. In order to provide a solution to the controversy, and in response to many requests from both side of the Atlantic, I determined to set up a video demonstration of what Brown could see through his microscopes. It has already been shown(10) that the microscope now in the collections of the Linnean Society of London can be utilized for critical microscopy, and this microscope was, by resolution of the President and Council, taken from the collections in order to carry out the experiments at my own laboratory. The attempts were based on a central premise:

That the result would show the phenomena observed by Robert Brown in a form as close as possible to the view he would have obtained in 1827.

Much interest was aroused in the project (11), though the aim was not always fully understood. The response to an outline of the experiment published in London (12) was to assume that image-intensification was the aim (13). It must be emphasised that this was not the case. The recreation of a pioneering experiment can only be recorded if it offers today's observer sight of what the original researcher could have seen. A modern glass slide may be preferred to a mica slip; a pearl bulb may be more convenient than an oil-lamp; a platinum loop may be more easily available than a Victorian dissecting needle; and any of these substitutions may be made if expedient.

` What cannot be done is anything that provides a change in the appearance of the specimen as perceived by the observer. In obtaining micrographs of van Leeuwenhoek's specimens, utilizing the original microscope at Utrecht, considerable pains were taken to use unmounted and unstained material, just as Leeuwenhoek would have done. In subsequent reconstructions of early experiments involving simple [i.e. single-lens] microscopy, micrographs were obtained of living bacteria and yeasts. Each specimen was mounted on a standard 3" x1" slide and constrained by a coverslip. But there was no attempt at interference with the image generated by the lens.

In the case of the Brown experiments, the single #1 lens was mounted on a modified objective tube of a Leitz microscope. The video camera, recording directly onto 8mm tape, was mounted ready to record the resultant images. No microscope eyepiece or field lens was used in the light path. The *Clarkia* pollen was obtained from anthers of *C. pulchella* at the Botanical Garden at Cambridge University, and pollen specimens from other species within the Oenotheraceae were also utilized. Exactly as Brown recorded, the experiments were carried out in the month of June and the pollen grains were mounted in water after removal from pre-dehiscent anthers. A 10μm graticule was recorded at the end of the demonstration in order to provide an on-screen calibration.

RESULTS

The phenomenon of Brownian movement was well resolved by the original microscope lens. Within the pollen grains, ceaseless movement could be observed. There is clearly no question of extraneous hydrodynamic phenomena in such a closed system, and evaporation-induced turbulence could equally be excluded. But this qualification is unnecessary to any seasoned microscopist: Brownian movement is instantly recognisable for what it is. The size of particles within the pollen was hard to estimate, though a working diameter of approximately 2μm could be offered. Correlated observations on colloidal systems in Indian ink and cow's milk show that particulate matter below this size can be clearly visualized by the Brown lens. A distinction may be made between *resolution* and *visualization* in this context (14), for it is possible for a lens to visualize structures beyond the strict definitions of resolution and this may have been a significant consideration during Brown's original demonstrations.

The video demonstration was prepared in time for Inter-Micro 92 at Chicago (15) and was exhibited for the first time to the delegates on Monday July 13, 1992. The response was enthusiastic: Robert Brown's claims were clearly no exaggeration. After 165 years, his pioneering observations and the clarity with which he assessed their implications were made available to modern microscopists.

Brown's own circle of acquaintances were a varied and interesting group. Among them were Sir Everard Home, who plagiarized (and later burnt) the great collection of Hunterian papers and who lost the Royal Society's collection of Leeuwenhoek microscopes. There was Thomas Horsfield of Pennsylvania, the eminent explorer and naturalist, to whom Brown showed the phenomenon, and it was also demonstrated to Peter Mark Roget, an edition of whose famous *Thesaurus* remains in print to this day. Also in the group was William Wollaston, who gave his name to one of the last types of microscope lenses to be used before the general availability of the achromatic lens. Robert Brown clearly loved to tease, as well as to inform, his friends; when an astonished Charles Darwin was first shown the demonstration of cytoplasmic streaming in the staminal hairs of *Tradescantia virginiana* - and asked what it was - he was told by Brown, "Ah, that is my little secret!"

The presentation of Brownian movement and its discovery to an audience at Inter-Micro would fit comfortably with Robert Brown's own aspirations. If a modern-day counterpart to Brown's social milieu were to be desired, then this distinguished gathering at Chicago would be one in which he would find himself perfectly at home. It is a fitting opportunity to offer again the sights that greeted Brown's own associates in the summer of 1827.

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REFERENCES

1: Deutsch, D. H., Did Robert Brown observe Brownian Motion: probably not. *Bulletin of the American Physical Society,* **36** (4): 1374, April 1991. Reported in *Scientific American,* **265**: 20, 1991.

2: [Report] Centenary of Robert Brown's Discovery of the Nucleus, Linnean Society account describes the instrument as "surprisingly simple, being little more than a dissecting-microscope." *Journal of Botany,* January 1932.

3: Ford, Brian J., Robert Brown, Brownian Movement, and teethmarks on the hatbrim [leading paper], *The Microscope,* **39** (3 & 4): 161-171, 1991.

4: King, P. P., Character and Description of *Kingia,* [in] *Narrative of a Survey of the Intertropical and Western Coasts of Australia*, **II**: 536-563, London, 1827.

5: *Ibid.,* Botanical Appendix: 534 *et seq.*

6: Ford, Brian J., The Controversy of Robert Brown and Brownian Movement, *Biologist*, **39** (3): 82-83, June 1992.

7: [in] Brown, R., A Brief Account of Microscopical Observations, etc., London (not published) 1827.

8: *Ibid.,* p 8.

9: Perrin, J. B., [in] *Annales de Chimie et de Physique,* 8me series, September 1909. Translated by F. Soddy F.R.S., as *Brownian Movement and Molecular Reality,* Taylor and Francis, London, 1910.

10: Ford, Brian J., The Restoration of Brown's first Botanical Microscope, *Microscopy*, **34:** 406-418, December 1982.

11: Bown, W., Brownian Motion sparks renewed debate, *New Scientist,* **133**: 25, 15 February 1992.

12: See reference (6) above.

13: Wheatley, D., [letter] Brown motionless, *Biologist,* **39** (4): 124, September 1992.

14: Ford, Brian J., The concept of 'Antipoint' applied to submicroscopic fibrillar structures, *Proceedings of the Royal Microscopical Society,* **3** (1): 14-16, March 1968. Ratification reported in: The proposal over 'Antipoint' [5th Report of Nomenclature Committee], *op. cit.,* **3** (3): 146, July 1968.

15. Ford, Brian J., [lecture] *An Evening with Brian: The Proof of Brownian Movement*. Inter Micro 92, McCormick Center, Chicago, Illinois, 2000h, 13 July 1992.