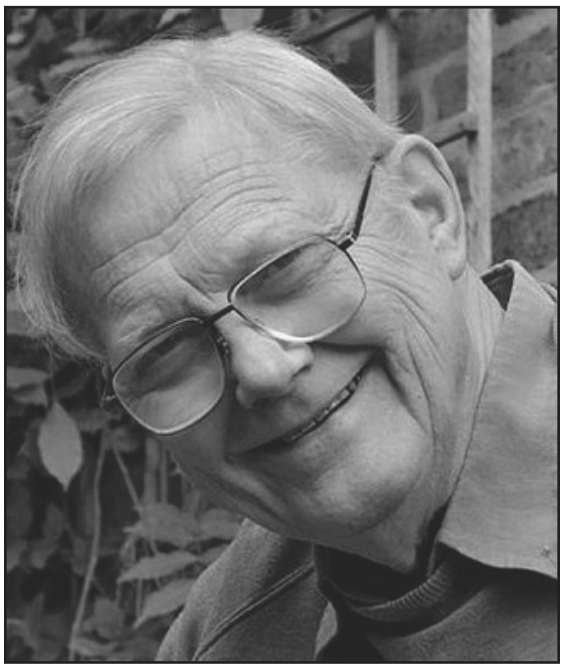
BRYAN CAMPBELL CLARKE



ANN CLARKE

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PROFESSOR BRYAN CLARKE was a world-leading evolutionary geneticist. He combined theoretical understanding of the principles of evolutionary biology, an appreciation of the process of molecular evolution, and a love of fieldwork, through which he studied the genetic diversity of wild populations and the patterns of natural selection that operated on them.

Bryan's primary interest was in studying evolution in the wild. In trying to observe evolution in action, geneticists focus on genetic polymorphisms, in which different genetic types ("morphs") coexist in the same wild population. In understanding how such variation is generated, and how it is maintained, we gain insight into the process of evolution as it has operated over the course of life on earth.

Bryan's early years were spent in England. His family had roots in the Bolton area of Lancashire—a county whose industrial legacy of cotton mills contrasts with its possession of some of the most pleasant rural areas of the country. But Bryan was born in the summer of 1932 in Gatley, a rural suburb south of the industrial city of Manchester, in the county of Cheshire. Later, age 6, Bryan moved with his parents and sister to the county of Northamptonshire, where he lived initially in the village of Stanwick, moving to Sywell after one winter. Their home at Sywell Hall, an Elizabethan house of 40 rooms, reflected the family's increasing fortunes. His father had a keen interest in all things scientific and stimulated Bryan's interests in fossils and microscopy. However, the coming of World War II broke up the family, and in 1940, Bryan was evacuated, surviving U-boat attacks on the convoy taking him, his sister, and his mother to Nassau in the Bahamas, after a short stop in Montreal. In Nassau, when Bryan was 8, they received tragic news—his father had been killed during a bombing raid that destroyed the Café de Paris in London's west end. Now short of money, and with Bryan and his mother without the visas that allowed his sister (on grounds of her asthma), to enter the United States, they moved to the Bahamian island of Eleuthera. Bryan's burgeoning love of natural history, particularly his lifelong passion for malacology, was encouraged by his 18 months on this tropical island. For the rest of the war years, he studied in Massachusetts, returning to school in Oxford, England, in 1945.

At 18, Bryan did his national service for two years in the Royal Air Force as a pilot officer and was sent to Canada to train as a pilot. Then, age 20, he went to the University of Oxford to read Zoology at Magdalen, acknowledged as the most beautiful of the Oxford Colleges. Bryan stayed at Oxford, and Magdalen, for his DPhil (as Ph.D. degrees are called at Oxford). Then, supervised by Arthur Cain, he started on the study of the visible polymorphisms in the common European snail *Cepaea nemoralis* and its close relative *C. hortensis*. *C. nemoralis*

played a large part in evolutionary genetics in the twentieth century, serving as a cornerstone of the field of ecological genetics, in which patterns of natural selection acting on genetic variants were identified as being the result of the species' ecological interactions.

Evolutionary genetics was a creation of the twentieth century. Charles Darwin, in elucidating his theories of natural selection in 1858–9, realized that selection could generate evolutionary change only if characteristics that were favored by selection were passed on to the next generation. And it was clear, from the artificial selection of characteristics by plant and animal breeders, that traits were indeed inherited. But Darwin was forced to admit that the mechanism through which traits were passed to offspring was quite unclear to him. Simultaneously, however, in his Moravian monastery garden, Gregor Mendel was carrying out the experiments that would establish the modern theory of genetics, the missing component of Darwin's theory of evolution by natural selection.

Following the re-discovery of Mendel's results by Hugo de Vries, Erich Tschermack, and Carl Correns in the early years of the twentieth century, the Mendelian theory of genetics and the Darwinian theory of natural selection were united through the so-called "neo-Darwinian synthesis," created in the 1920s by J. B. S. Haldane and R. A. Fisher in the United Kingdom and Sewall Wright in the United States. This synthesis generated a new science of the genetics of populations, which was appreciated as the key to understanding the evolutionary process. The neo-Darwinian synthesis was primarily a mathematical theory about ways in which natural selection would act on genetic variation in natural populations. But it was also appreciated that the frequencies of genetic variants in populations would change, over time, through a random process called *genetic drift*, which is particularly powerful in small populations. Among the architects of the neo-Darwinian synthesis, Fisher and Haldane, who believed that the numbers of individuals in wild populations were large (millions), attached less importance to genetic drift than did Wright, who envisaged populations that fluctuated in size but would often drop below a thousand. It was a remarkable feature of the early years of population genetics that the theoretical understanding of selection and drift (and also the creation of new genetic types by mutation) greatly preceded the identification of data sets that could be used to test the theory.

For experimental population geneticists, the testing and application of the mathematical theory of population genetics required the study of genetic variation in populations, in particular the study of polymorphisms. The easiest polymorphisms to study are those in which the genetic variation reveals itself unequivocally in the appearance of the

organism, so-called "visible polymorphisms." *Cepaea nemoralis* is a treasure trove for students of visible polymorphisms. Shells can be banded or unbanded and can have one, three, or five bands, with these characteristics controlled by two variable genes. The background color of the shells also varies, with pink, yellow, and brown possibilities, controlled by a third variable gene. The genetics of this variation was rapidly elucidated: different variants (called alleles) at one gene determine the presence or absence of bands on the shell, different alleles of another gene determine the number of bands, and different alleles of yet a third gene determine the shell color. Thus, a glance at a snail shell revealed the animal's alleles at these three genes. Indeed, genetic variation in other genes also affected the snail's shell patterns (for example, whether the lip of the shell was dark or light).

Visible polymorphisms allowed the study of the forces acting on genetic variation. In early studies, it had been suggested that it did not matter to a snail whether it had bands or what color it was, that this variation was not acted on by Darwinian natural selection (being thus described as "neutral" variation). But work by Philip Sheppard and Arthur Cain demonstrated that natural selection was indeed triggered by these differences in appearance between snails. The frequencies of morphs showed a strong relationship with the environment, consistent with shell colors and patterns being influenced by their crypsis or distinctiveness to predators on different backgrounds. Bryan Clarke, in his doctoral work at Oxford and subsequently, found further evidence for natural selection acting on the *Cepaea* polymorphisms and did so by his inclusion of a related, coexisting, and equally polymorphic snail species—*C. hortensis*—in his studies. The patterns of correlation between variations in the two species suggested that predators were hunting both species simultaneously and showing the same preferences in each for particular bands and colors in their choice of prey.[1,2] Bryan always regarded Philip Sheppard as a key mentor, and one of the most incisive minds working in evolutionary biology; Sheppard died in 1976, cruelly early at age 55.

Through the work of Sheppard, Cain, and Clarke, it became generally accepted that visible polymorphisms in *Cepaea*, and indeed those in other genera, were subject to natural selection. But if the question that these snails were being used as a model system to answer was whether genetic variation was typically affected by natural selection rather than simply by genetic drift, there was a caveat. *C. nemoralis* was chosen for study simply because it showed extraordinary levels of visible polymorphism. Could the results obtained with this unusually polymorphic species be extrapolated to more typical species in the wild, which did not show obvious polymorphic variation? Could they be extended to the

fruit fly *Drosophila*, the geneticists' main tool? In 1966, techniques were published that allowed the identification of genetic polymorphisms in every species—polymorphisms with no visible effect but which were detected by observing the electric charge on soluble enzymes.[3,4] Proteins from the organism were separated by gel electrophoresis, where their mobility depends on the molecules' electric charge. Charge differences are caused by substitutions of charged for uncharged amino acids in the protein, reflecting genetic differences. Individual proteins were identified from the thousands of protein species in the extracts used by virtue of their specific enzyme activities. Using this technique, no longer did the study of genetic variation in the wild require visible polymorphism of the *Cepaea* type—all species turned out to have at least some protein polymorphisms that could be studied electrophoretically. But this re-kindled the question that had been unequivocally answered for visual polymorphisms: Is this variation in the charges of enzymes affected by natural selection, or is it neutral?

The widespread use of gel electrophoresis to study these "allozymes" (allelic states of enzymes) triggered an extraordinary period in which evolutionary biologists from the world community held widely opposing views. Bryan Clarke was a key figure in the "selectionist" camp. He believed that the variation in the charge of enzymes, as with the *Cepaea* visible polymorphisms, indeed affected the organism's ability to survive and reproduce. In other words, this kind of variation was subject to natural selection. "Neutralists" approached this protein variation hypothesizing that the biology of the organism and, more specifically, its Darwinian fitness were unaffected by the amino acid differences that caused these measurable charge differences. They argued that this form of protein variation was affected only by the random forces of mutation and genetic drift. The theories were fundamentally different, but it was very hard to get the organisms to reveal which was correct. It seemed that each data set could be interpreted either in a selectionist or neutralist way. One way forward, which Bryan Clarke pioneered, was to study the enzymology of these protein variants: Did they have different values of K_m and V_{max}? He and others found that generally yes, they did.[5,6] But did this mean that natural selection could distinguish between the alleles? The action of natural selection on these enzymes was harder to study. The questions that were being asked were empirically challenging; if one genetic type had a 1% higher chance of surviving to adulthood in the wild, this strength of selection would be powerful enough to have a major influence on the evolutionary trajectory of a species. But identifying a difference of 1% in the probability of survival in the wild with statistical confidence is virtually impossible. The neutralist–selectionist debate, initially highly controversial, tended to become quieter as

techniques capable of resolving the issues were awaited. More recently, powerful DNA evidence, looking at variation in the sequences of the genes that encode polymorphic enzymes, is typically revealing signs that selection has indeed acted on these alleles.

There is, of course, a fundamental question that has to be addressed if a polymorphism is acted on by natural selection. If one type, be it a visible variant in a snail or a protein variant in a fly, has a higher chance of surviving and reproducing, one would expect the frequency of that variant in the population to inexorably increase, until it reaches 100% (described by population geneticists as it being "fixed"). In other words, selection should destroy the polymorphism. Thus, we need to ask (and this was, for Bryan, a fundamental question): How can natural selection maintain, rather than destroy, variation? In the 1950s, as a result of studies of the sickle cell polymorphism at the beta-globin gene in man, the favored hypothesis for the selective maintenance of polymorphism was heterozygote advantage. As every individual has two copies of each gene (one from each parent), if two different alleles exist in the population, an individual can have two copies (said to be "homozygous") of one allele, or two copies of the other allele—or they could have both alleles (a "heterozygote"). Heterozygote advantage is the situation where the heterozygotes have a higher ability to survive and reproduce than either homozygote. Godfrey Hardy and Wilhelm Weinberg had, in 1908, demonstrated that the relative probabilities of homozygotes and heterozygotes in a randomly mating population are predicted by the binomial theorem, and this result has the consequence that any rare allele will almost always be heterozygous. Students of polymorphisms appreciated that this means that, with heterozygote advantage, the net success of an allele increases as it becomes rare, and selection can maintain a stable equilibrium in which two alleles will persist in a polymorphic state.

However, Bryan Clarke looked for a cause of stability of polymorphisms that was not a consequence of the genetic system but was created by organisms' interactions with their environments. He identified situations in which the relative fitness of individual genetic types varied with their frequencies in the population. In this "frequency-dependent selection," variants do better when they are rare. But why should this happen? Bryan identified one mechanism, which he called *apostatic selection*, a phenomenon in which predators hunting visually for polymorphic prey will tend to take disproportionately more of the commoner form, thereby giving an advantage to rare morphs. He was able to establish, initially in his 1968 *Nature* paper with John Allen,[7] that this process was highly repeatable, identifying the birds and other predators that selected color and banding morphs in this way, with its consequence of stabilizing the frequencies of morphs in the snails' polymorphism. Bryan was also able

to demonstrate, in his *Nature* paper with Ian Soane in 1973,[8] that the same happens when mammals hunt their prey using olfactory cues. In his 1979 *Nature* paper with Fred Allendorf,[9] he also postulated that frequency-dependent selection would be expected with allozyme variation as well if the enzymatic properties of the enzymes produced by different alleles were different.

Although the electrophoretic study of soluble enzymes first permitted the study of polymorphisms at the molecular level in the 1960s, the development of DNA cloning and sequencing technologies, and, in particular, the polymerase chain reaction in 1985, switched the focus of molecular population genetics from protein sequences to DNA, with the genetic code being used to predict the amino acid sequence of a protein from the base sequence of its gene. The use of DNA information created a common currency that could be used to compare alleles within populations as well as compare genes between species. And in the molecular evolutionary process that created differences in genes between species, a precisely analogous question to the neutralist–selectionist debate concerning allelic variation immediately suggested itself: As proteins evolve over the longer term, what proportion of amino acid changes are driven by natural selection, and what proportion are neutral mutations spreading to fixation through genetic drift? Bryan Clarke was a champion of the view that the majority, perhaps almost all, of the amino acid changes that take place over evolutionary time are selectively driven. An opposing view came from Motoo Kimura, from the National Institute of Genetics at Mishima, Japan. Kimura's book *The Neutral Theory of Molecular Evolution* (1983),[10] was a classic exposition of the ways in which the data of molecular evolution could be accommodated by a theory in which almost all molecular changes occurred through a combination of neutral mutation and genetic drift, with just a tiny fraction of the molecular changes being responsible for the adaptive phenotypic changes that differentiate populations and species. One key element of the theory of neutral evolution is that the process should occur at equal rates, whatever the size of a population. So, when a so-called "molecular clock" was found, with amino acid and DNA sequences changing at similar rates in different lineages, this clock-like behavior was identified as supporting the neutral theory. The molecular clock issue was a key episode in the history of the neutralist–selectionist debate, with selectionists such as Bryan observing that the clock-like nature of molecular evolution was far from perfect and stressing that there are ways in which selectively driven evolution can also end up being clock-like. One of the compelling features, for a young scientist such as me, of these deep divisions was that almost always, the debates did not

became personal and were carried out in an atmosphere of mutual respect. Bryan's gentlemanliness and ability to appreciate the sincerity and worth of his opponents' positions was a key factor in the maintenance of this civilized tone.

Bryan's academic progress was rapid. He was appointed as an Assistant Lecturer at the University of Edinburgh in 1959 and was Reader when he left in 1971 to establish the Department of Genetics at the University of Nottingham, which is where I later knew him well following my appointment in 1987; we remained friends after he became a Professor Emeritus in 1997. In 1960, he married Ann Jewkes, who survives him; he is also survived by their daughter, Alex and son, Peter. He met Ann, the daughter of Oxford classical liberal economist John Jewkes, at the Oxford Zoology department, where she was working with the future Nobel Prize winner Sir John Gurdon.

Bryan advocated, from very early on, that one way to investigate evolution most directly was to observe evolutionary processes taking place in the laboratory, and appreciated that, to have sufficient numbers of individuals and enough generations for significant evolutionary change to happen over the timescale of a specific research project, doing so required the use of microorganisms. Such "experimental evolution" studies are now being carried out in laboratories throughout the world, using bacteria and eukaryotic microbes.

Bryan was aware that genetic drift is just one of the random forces that can operate in evolution. In collaboration with G. S. Mani, he also investigated the phenomenon that he called mutational order.[11] This study concerned the introduction of stochasticity into evolutionary change through the randomness of mutation. Bryan examined the situation where adaptation required an evolutionary change at a single gene, but in which any number of genes could be the one where the adaptation took place. In this situation, genetic drift would play some role in deciding which gene was the one where the change took place, but equally important would be the random element of which gene was the one that received the advantageous mutation.

In the second half of his academic career, Bryan's experimental work mainly concerned the snails of the genus *Partula* on the islands of the South Pacific, notably those on Moorea, in the Society Islands. This work was a long-term collaboration with Ann Clarke and with Michael Johnson of the University of Western Australia and Jim Murray of University of Virginia. The prime focus of this research was in catching the process of speciation "in the act." Seven species of *Partula* lived on Moorea, and more than 100 other species were found on other Polynesian islands. *P.suturalis* and *P. taeniata* were found throughout the island, whereas *P. mooreana*, *P. tohiveana, P. exigua*, *P. mirabilis*, and *P. aurantia*

had more restricted distributions. Two other species identified by earlier researchers, *P. dendroica* and *P. olympia*, were shown by Clarke and Murray to be races of *P. suturalis* and *P. tohiveana*, respectively. All species are visibly polymorphic, including, in *P. suturalis*, a polymorphism in the direction of the coiling of the shell (left- or right-handed).

All of these species, except *Partula exigua*, were brought to the United Kingdom and maintained as captive populations. Very regrettably, the carnivorous snail *Euglandina rosea* (the rosy wolfsnail from central America) was introduced to Moorea as a biological control agent for the invasive giant African land snail *Achatina fulica*, but it ate the indigenous *Partula* species. Bryan had to report, in his paper in *Pacific Science* in 1988,[12] that all seven *Partula* species on Moorea were now extinct in the wild. However, the studies on the biology of the species continued with captive-bred and preserved specimens. The species, including the two widespread and sympatric species *P. suturalis* and *P. taeniata,* are quite distinct from each other in their morphology and behavior. Although all of the species are clearly closely related and share recent ancestry, a key question is whether they are still exchanging genes, or whether they are reproductively isolated and thus good biological species. Work by Clarke, Murray, and Johnson in 1996,[13] studying allozyme variation, revealed a remarkable geographic structure, in which these two species showed similarities in their allozyme patterns at particular geographic locations, a result that demonstrates the flow of alleles between the species on a local scale. So it was possible for ecologically diverged but sympatric species to exchange genes while maintaining distinct morphologies and behaviors. This insight, that molecular variants may give a false picture of the differentiation between species at more selectively important genes, was ahead of its time. Since then, much more extensive work, using genomic sequencing, in a number of species is revealing that the early stages of speciation may indeed be accompanied by gene flow, and that genes involved in maintaining the morphological and ecological distinctness of incipient species may form genomic islands of divergence, surrounded in the genome by genes that are homogenized between the species by the flow of genes that arises from hybridization.

Bryan Clarke had many scientific honors. He was elected a Fellow of the Royal Society in 1982, an International Member of the American Philosophical Society in 2003, and a Foreign Honorary Member of the American Academy of Arts and Sciences in 2004. He was Vice President of the Genetical Society from 1981–3. Medals and awards include the Linnean Medal for Zoology in 2003, the Darwin-Wallace Medal of the Linnean Society in 2008, and the Royal Society's Darwin Medal in 2010. He had countless invitations to speak at international meetings and serve

on editorial boards, and he edited the journal *Heredity* from 1978–85 and the *Proceedings of the Royal Society Series B* from 1989–93.

He also played an important role in the administration of genetic research in the United Kingdom. Notably, he chaired the Biological Sciences panel of the 1996 Research Assessment Exercise, whose quality assessments drove university research funding from the government. In his work on the national stage, he was a constant advocate for the importance of pure, curiosity-driven research in science, in a world where pressures on scientists, notably from governments, were constantly seeking to divert them into more commercially oriented studies. One of Bryan's many enduring legacies was the founding in 1968 of the Population Genetics Group, a series of relaxed, friendly, but scientifically rigorous, meetings that gave many young graduate students (including me) their first chance to present their work at a scientific conference. These meetings continue to thrive, and the 50th meeting took place in Cambridge in January 2017. At the first meeting of the Population Genetics group following Bryan's death (held at Sheffield in January 2015), I had the honor to present a eulogy to Bryan at the start of the meeting.

The threat to *Partula* in the wild was recognized early, and methods were developed, particularly by Vivian Frame at the University of Nottingham Genetics Department, to breed the species in captivity; populations were also established in numerous zoos. But the loss of *Partula* in the wild (and attempts at re-introduction have not succeeded) demonstrated the vulnerability of species, including invertebrates, to environmental change. In 2002, Bryan and Ann Clarke, with embryologist Dame Anne McLaren, founded The Frozen Ark, a charity that seeks to preserve DNAs, cell lines, and gametes of endangered species. The charity has established a consortium of more than 20 zoos, biology institutions, and museums worldwide, dedicated to ensuring that even if a species becomes extinct, the study of its genes can continue, with the possibility of its renaissance through nuclear transfer being envisaged as molecular and cell biological techniques advance. The work of the Frozen Ark continues today.

Bryan's remarkable scientific achievements were mirrored by his human qualities. He had little time for university bureaucracy, but in the department he ran created a stimulating and inspiring atmosphere in which all scientists, including the most junior, were encouraged to talk about their results and interests. He was a conspicuously kind, charming, and entertaining man who continues to be greatly missed in the evolutionary genetics community throughout the world.

Elected 2003

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ENDNOTES

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