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The making of a moth man

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At the outset of my own career, I sought to exploit a boyhood enthusiasm for Lepidoptera natural history to help design evolutionary experiments. Geneticist colleagues and friends first in Zoology at the University of Texas, and later in the Galton Laboratory of Genetics and Biology, University College London, would often try to persuade me that I'd be better off switching to a more tractable or better studied genetic system, such as *Drosophila* or humans. I stubbornly refused, perhaps unwisely, and carried on with butterfly and moth research regardless.

Bruce Grant on the other hand, went in the opposite direction. He left lab organism genetics (*Drosophila*, and the tiny parasitoid wasp, *Nasonia*), and became an expert on moth trapping and moth breeding halfway through his academic career, to work on industrial melanism in the peppered moth *Biston betularia*. In this engaging book, Grant documents the places he went and the extraordinary people he met, on this quest to understand parts of the peppered moth story that did not quite add up.

Twenty years ago, Bruce Grant retired from his Professorship in Biology at the prestigious William and Mary liberal arts College in Williamsburg, Virginia. Grant was clearly hugely influential there in his teaching of genetics and evolution; many of his undergraduate students are among today's most renowned evolutionary biologists. Most of these were first drawn to research questions by Grant's lectures, his personal encouragement, and through work in his laboratory. In his first Spring semester, he inspired Jerry A. Coyne's interest in speciation. He served on the thesis committee of the young molecular ecologist, Walter F. Eanes. Gregory A. Wray, who later came to fame with the rise of evo-devo, first did research with Grant as an undergraduate on *Nasonia*. H. Allen Orr was a philosophy major until he took Grant's course on evolution, and joined his lab, later becoming with Jerry Coyne one of the two major figures in the study of speciation of our times. Norman A. Johnson, the book review editor of this very review, who is now at University of Massachusetts, Amherst, became another speciation enthusiast inspired by working in Grant's laboratory at William and Mary. Mohamed A.F. Noor also came under Grant's spell in his undergraduate days, and is now a celebrated evolutionary biologist who became the Editor in Chief of "Evolution," and is Professor of Biology at Duke University. I've known their work and met all of these well-known evolutionary biologists, though I've never met Grant himself. A tribute to Grant on his retirement, celebrating his influence on the course of evolutionary biology, has been written by two of his well-known academic progeny (Noor and Johnson 2005).

Most readers will have heard the basic British peppered moth story, I think. I learnt it in high school, but I'd read about it long before in a book, "Moths" by E.B. Ford, that my father had in his library (Ford 1955). The peppered moth in the early 19th Century was typically white sprinkled with black, so explaining its name. It is a geometrid (inchworm), and the adult moth, like many others in its family, rests on surfaces with its wings spread out flat. It hunkers down, pressing its wings to the surface, so that it can become shadowless and almost invisible on any pale surface such as a tree trunk or tree branch: the black speckles are more conspicuous than the outlines of the wings, which helps the moth blend into the background. The industrial revolution in 19th Century Britain changed things: black soot from coal smoke began to cover walls, trees, and virtually any surface near major urban and industrial centers. The pale speckled pattern of the peppered moth now revealed its outline, rather than concealing. Rare mutant forms first detected in the mid-19th Century had wings and body all black, and were far less conspicuous on the soot-polluted surfaces than the pale morph. The melanic form quickly became abundant, and by the end of the century was the predominant morph near industrial cities like Liverpool,

Manchester, Birmingham, and London. The author of an early moth book (Tutt 1896) explained this as a result of natural selection mediated by visually hunting predators, particularly birds. It was later discovered that melanism is inherited at a single locus, and that the melanic allele is dominant. J.B.S. Haldane used the documented rapid rise in allele frequency of the melanic allele between 1848 and 1901 as an example for his mathematical genetic theory of natural selection. He showed that the melanics must have benefited from a massive ∼50% higher fitness than the typical speckled form to explain such a rapid rise in melanism (Haldane 1924).

In the 1950s and 1960s, the British ecological geneticist Bernard Kettlewell carried out field experiments to test whether bird predation was indeed the likely agent of selection. By means of mark-release-recapture experiments, he documented strong selection pressures, similar to Haldane's estimates, for and against melanism in polluted and unpolluted woodlands (Kettlewell 1955b, 1956). With Niko Tinbergen, he observed and filmed birds attacking moths on different backgrounds, showing that birds were indeed likely predators. Similar experiments have since been replicated many times by different workers at many different sites, and bird predation and camouflage is today generally vindicated as a general explanation for melanism in the peppered moth and many other species, in spite of ill-founded criticisms and doubts raised in the 1980s to early 2000s (Lees 1981; Cook 2003; Cook et al. 2012). I've summarized the backdrop story here for completeness, but this much you probably already knew. Grant, like most other evolutionary biologists, had taught the peppered moth story in his undergraduate lectures.

However, the main part of Grant's book, and story of his own work with peppered moths, instead covers remaining difficulties that earlier work had left unsolved. To simplify, peppered moths were either black (melanic) or pale (speckled). Most populations, even the most melanic or the most rural, are somewhat polymorphic for black and white forms. But a pale speckled typical form resting on a sooty background would get attacked, as would a melanic on a pale background. How did the ill-adjusted moths survive in these polymorphic populations? One idea was that moths chose their backgrounds carefully to match their prevailing wing pigments.

Kettlewell had done experiments in "large cider barrels" (British readers will recognize that these were barrels for hard cider). He put black and white paper strips on the interior sides of the barrel, and then put the barrel outs of doors with the top covered in muslin. He let three melanic and three pale (speckled) morph moths settle in this apparatus in each barrel overnight. Melanics tended to settle on black paper, and typical morphs on white paper with a bias, in each case the ratio was approximately 2:1 in favor of the closest matching background (Kettlewell 1955a). Subsequent work did not hold up, and Grant smelled a rat. In particular, Kettlewell's preferred explanation for his results, that the moths somehow compared their own hue to the background, seemed unlikely and did not have any evidence. Kettlewell argued that the moths looked over their shoulders, so to speak, and wandered around to match their wings to the background. When British ecological geneticist Jim Murray showed up at Williamsburg to give a lecture on snail polymorphism, Grant confronted him on this obvious difficulty with Kettlewell's result: "Why doesn't someone just do those experiments over again, and this time do them right?"

Murray replied, "Why don't ***you*** do them?" This single comment seems to have led to the *volte-face* in Grant's research program. Murray ran the University of Virginia Mountain Lake Biological Station, at the other end of Virginia, and was keen to recruit fieldwork there. Murray told Grant that David A. West, from Virginia Tech, had been moth trapping there for many years, including many peppered moths. Because the peppered moth story is normally told about British peppered moths, many people do not realize that the same species (the subspecies *Biston betularia cognataria*) occurs also in the United States.

As an aside, I knew of James Murray, the author of an irreverent slim volume that I bought in 1973 and which informed my own 1970s undergraduate career, long before I'd ever heard of Bruce Grant. The likely importance of gene flow and parapatric speciation was clearly outlined (Murray 1972). David A. West, meanwhile, had a long and varied career working mainly on Lepidoptera genetics and evolution, especially with Papilionidae (swallowtail butterflies). West's research on Papilionidae in Brazil also led him in his last days to write the definitive biography of a major correspondent of Charles Darwin, Fritz Müller, the discoverer of mutualistic (Müllerian) color pattern mimicry (West 2016).

In the course of doing those experiments, Grant developed an ingenious experimental technique for testing Kettlewell's hypothesis for visual background matching, by placing white or black paper collars around the necks of live moths so that they could not see their own wings. Elizabethan moths! They look a little angry about this (see Fig. 1)!

Without giving any punchlines away, by means of experiments Grant was able to show that American peppered moths, at least, did not behave in the manner that Kettlewell had asserted. But at Mountain Lake, there were also few melanics. He realized that he needed to go to Britain to perform similar experiments there.

Grant wrote to various experts on the peppered moth in the United Kingdom, including the British academics David R. Lees and Laurence Cook, but they were mostly gloomy and discouraging, as British academics tended to be (especially back then, in the early 1980s, when Margaret Thatcher was Prime Minister and government cuts led to scarce funding for science).

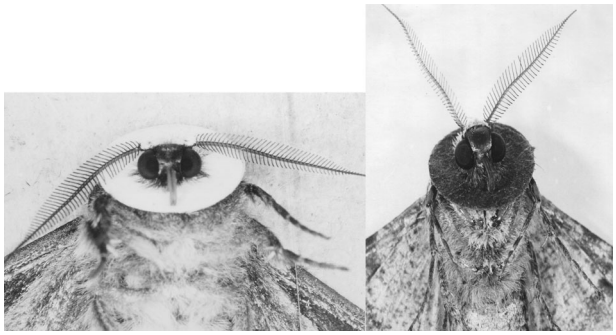

Figure 1. Grant's "Elizabethan Moths:" his technique of placing paper collars on moths so that they could not see their own color patterns (Photo © Bruce Grant).

His third contact, however, Sir Cyril Clarke, was much more forthcoming; he had trapped moths near Liverpool every year since 1959 and had caught 689 peppered moths during the previous year, of which 445 were melanics. Sir Cyril was a physician who had been studying genetics of butterflies as well as humans with Philip Sheppard. Clarke was knighted for his work that developed a successful treatment for the clinical problem of Rhesus blood group incompatibilities in the human fetus, which until the work of Sir Cyril's group in the 1960s was a persistent problem for a significant fraction of pregnancies. The Rhesus complex of antigens are products of a tandemly duplicated gene, and Sir Cyril claimed that his work with butterfly "supergenes" had led him to his idea for treating the Rhesus problem.

Sir Cyril was very welcoming, with typical enthusiasm: he cultivated a worldwide network of fellow enthusiasts and collaborators for butterflies, moths, genetics, and evolution, and was keen for Grant to come to stay; he also pointed out that he needed people to crew for him on the 18-foot yacht he raced in the Dee Estuary. I met the Clarkes a year after Grant did, when I was planning a trip to Peru to study *Heliconius* butterflies (he also had a colony of *Heliconius* in his Liverpool greenhouse). He was always dashing up and down between London and Liverpool on the train, constantly attended by his wonderful wife Lady Féo Clarke. Grant went to meet the Clarkes at their house in West Kirby, on the Wirral Peninsula near Liverpool, in 1984. He describes the Clarkes perfectly, and with great warmth and sense of humor, for example, the fact that Féo was incredibly important to Sir Cyril; she was essentially his accessory memory. Grant was able to use the Clarkes' moth trap in their garden, conveniently next to a wild patch of land, Caldy Common.

One of the charming features of this book is its concentration on nonscientific aspects, and his impressions of foreign places, like 1980s Britain. In Liverpool, he joined a pipe band and with them busked with his bagpipes in Liverpool pubs. He describes buying an "old banger" for £100, and having a fender-bender with it as he drove it away on the first day. He describes the Clarkes' household, with all the willing volunteers and workers on Sir Cyril's various butterfly and moth projects. Grant went back to America having thoroughly enjoyed himself. Furthermore, over the summer, he trapped plenty of moths for his experiments, and was able to smuggle British pupae to the United States for further experiments.

In 1986, he returned to the United Kingdom and this time met Rory Howlett who he discovered had been doing parallel experiments on background matching behavior for his Ph.D. at University of Cambridge. Although each was worried about being scooped by the other, they quickly became friends, and ended up amicably publishing the work together (Grant and Howlett 1988). We should all learn by this!

By the mid-1980s, rapid changes in morph frequencies of the peppered moth were happening. Sir Cyril Clarke had the best dataset from West Kirby going back to 1959, but Grant made important contributions by trapping near Detroit, Michigan, where Denis Owen (another British lepidopterist and ecological geneticist) had been trapping largely melanic peppered moths in 1959–1962. Due to clean air legislation, melanism began to be selected against, and there were precipitous declines in melanic frequencies near urban centers in both North America and Britain at this time. Grant and Owen were together able to put the comparative story together by trapping moths again in Michigan in the 1990s.

In the slides of a talk by Hiro Asami, a Japanese ecological genetics student of Jim Murray, Grant recognized the outline of a peppered moth. He had not realized they occur in Japan. Although Hiro thought that melanism was unknown in Japanese peppered moths, Grant resolved to try to find out if this was true; a three-continent comparison would be even more important and convincing evidence for natural selection. He travelled to Japan, and looked for peppered moths. He did find peppered moths, but very few, and there were no melanics. But of course, Grant seems to have had a lot of fun over 11 chapters describing this quest, finding out about Japanese customs. He describes the kindness of his Japanese hosts and his own impression of the language and culture in a most delightful way. To this day, only one melanic peppered moth has ever been caught in Japan, and this was after he got back to the United States. But Grant seems not to have minded.

The remainder of the book discusses some more recent controversies over the evidence for selection, the evolution of dominance, and Grant's own part in putting the controversies to rest. He also describes how continued research in Liverpool by Ilich Saccheri's group has led to the characterization of the melanic allele. In the United Kingdom, melanics all share a single large transposable element insertion (van't Hof et al. 2016) within a gene known to control color pattern in other Lepidoptera, including *Heliconius* butterflies. All this is told with typical Grant charm.

A memorable passage in this book for me is when Grant tries to explain to a nonacademic why he works on moths; he simply finds the genetics and evolution of moths the most interesting thing to study. That's why.

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